From Toxicity Testing in the 21st Century to New Approach Methodologies (NAMs) and Next Generation Risk Assessment (NGRA): Making Safety Decisions Without Harming Animals

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07/11/2022

Paul L. Carmichael

- First degree in Biochemistry (Toxicology)
- PhD in Biochemistry from King's College
- Postdoctoral Scientist in carcinogenesis and genotoxicity
- Senior Lecturer Faculty of Medicine at Imperial College
- Toxicology Senior Science Leader, SEAC, Unilever
- Academic links at Brown, Peking & Wageningen Universities

Web Resourse

- SEAC’s Website for what we are discussing today:

www.TT21C.org

Ensuring Safe Ingredients for Foods, Drinks, Homecare and Cosmetic Products

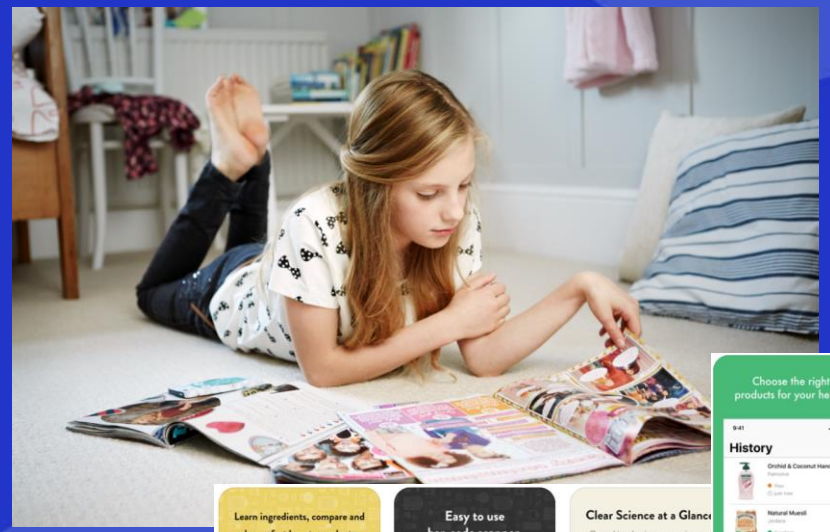
Risk Based Approach:
Considers both the hazard and the exposure to evaluate the risk

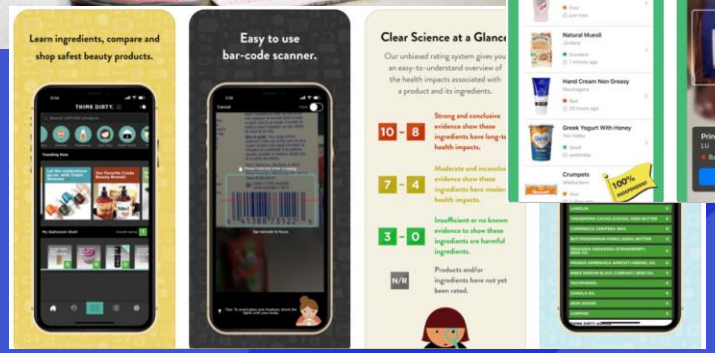
Can we safely use % of ingredient in product?

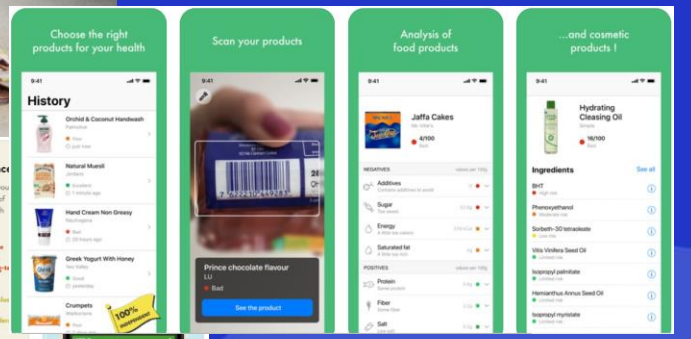
For **consumers**; **workers**;
the **environment**

All Consumers Want Safe Products But Majority Want Them *Not Tested On Animals* + Transparency

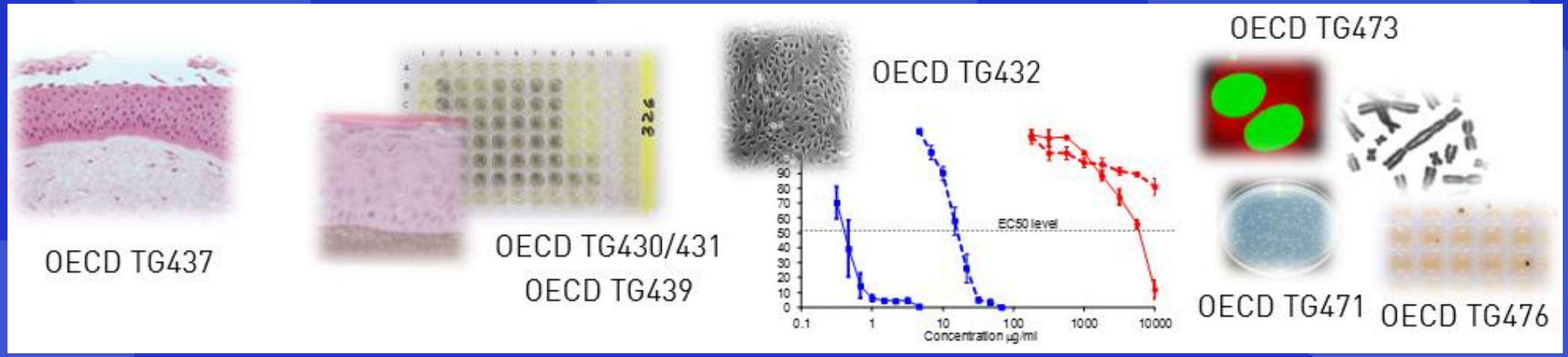
PETA APPROVED
Global Animal Test Policy





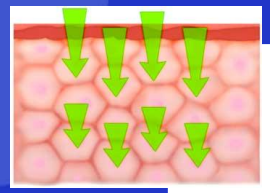


Use of Existing OECD *In Vitro* Approaches

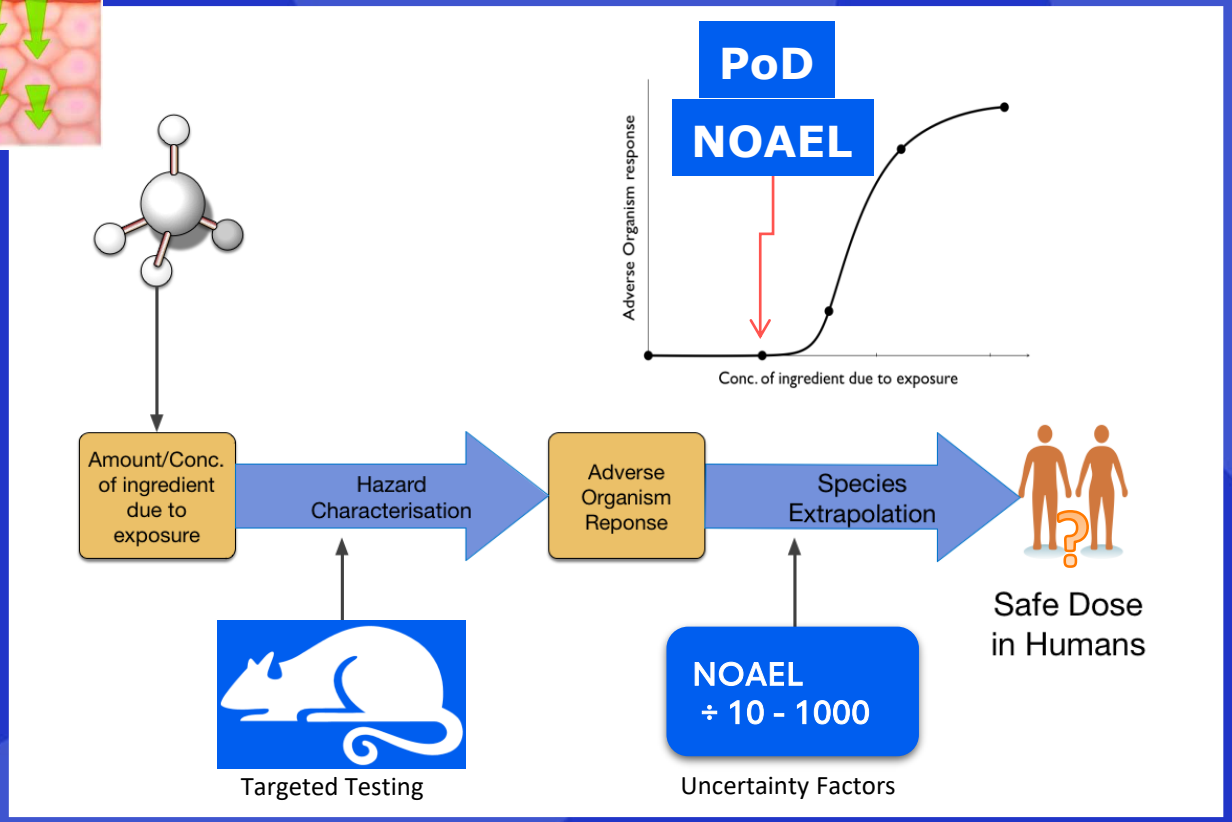


Skin and eye irritation; skin sensitization; phototoxicity; mutagenicity

But What About Systemic Toxicity?



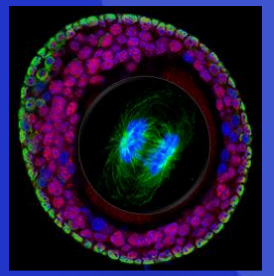
Is it safe?



e.g. 90 Day Repeat Dose Study

It has served us well enough

Mechanistic? Human-based?



Acute toxicity studies (LD50) developed

Repeat tox studies developed with FDA factors of 100

Genotoxicity assays developed

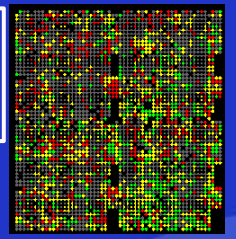
Studies for reproductive toxicity

1920 | 1930 | 1940 | 1950 | 1960 | 1970 | 1980 | 1990 | 2000 | 2010

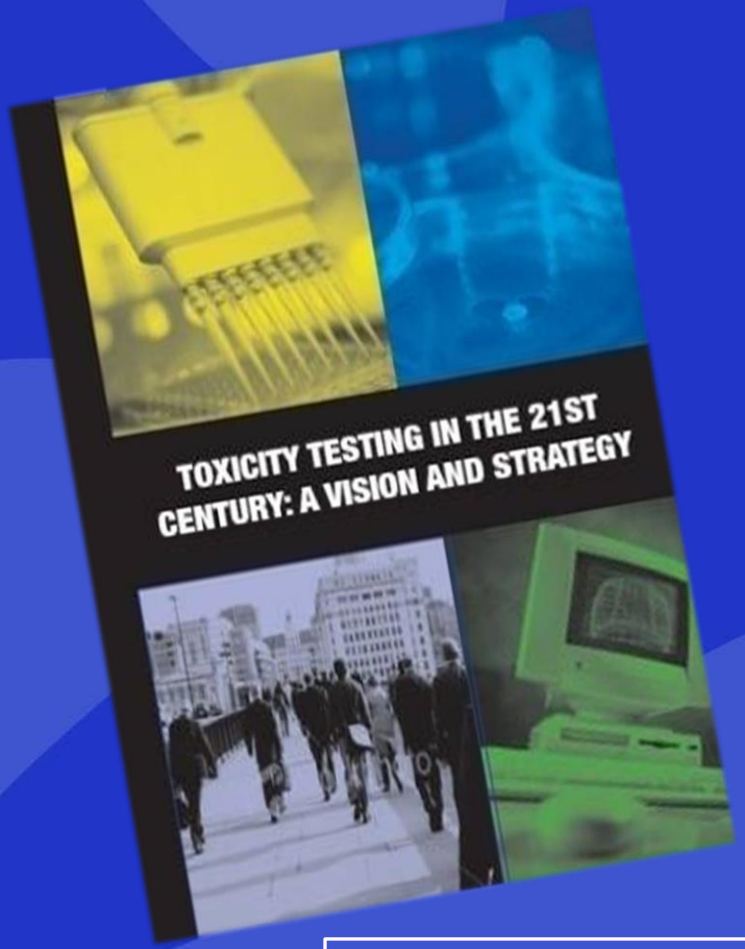
Draize test introduced for eye irritants

Thalidomide led to development-al tox tests

Rodent cancer bioassay



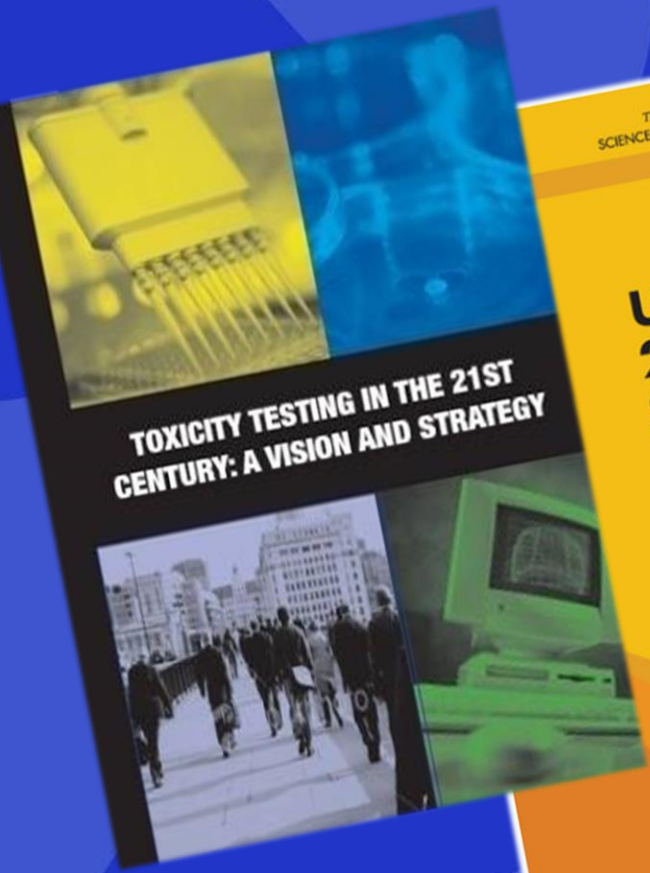
2007 Toxicity Testing in the 21st Century (TT21C)

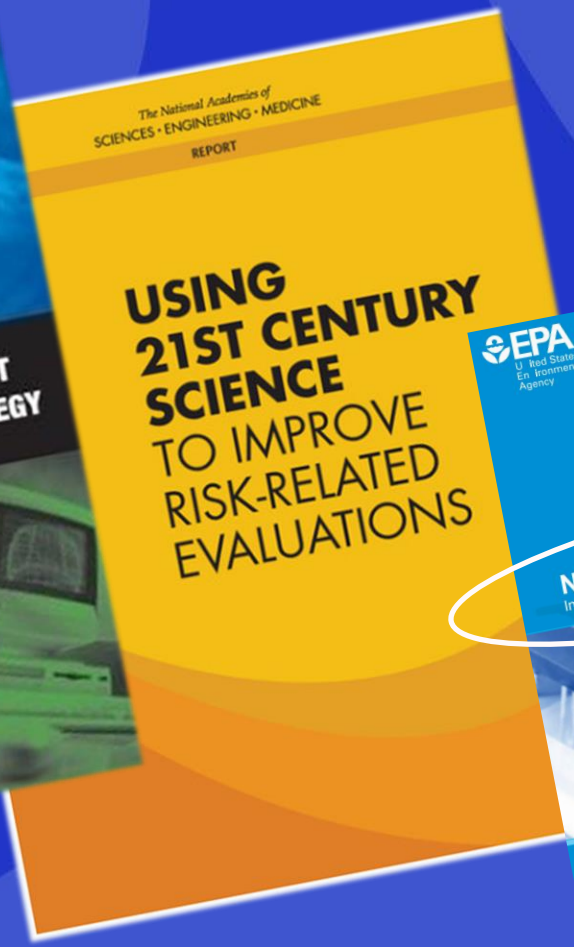


"Advances in toxicogenomics, bioinformatics, systems biology, and computational toxicology could transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin."

Perturbation of 'toxicity pathways' and stress responses

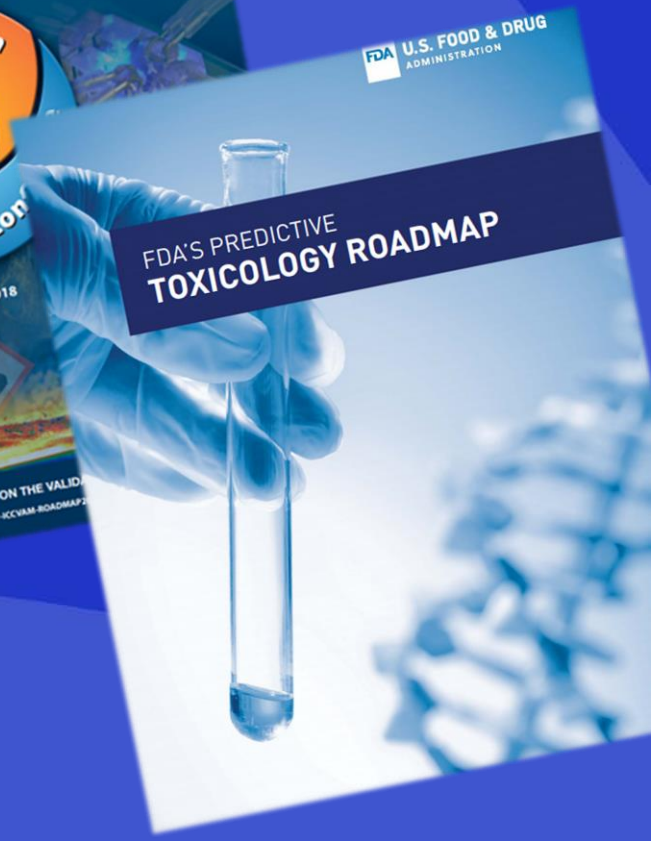
TT21C + NGRA


TOXICITY TESTING IN THE 21ST CENTURY: A VISION AND STRATEGY

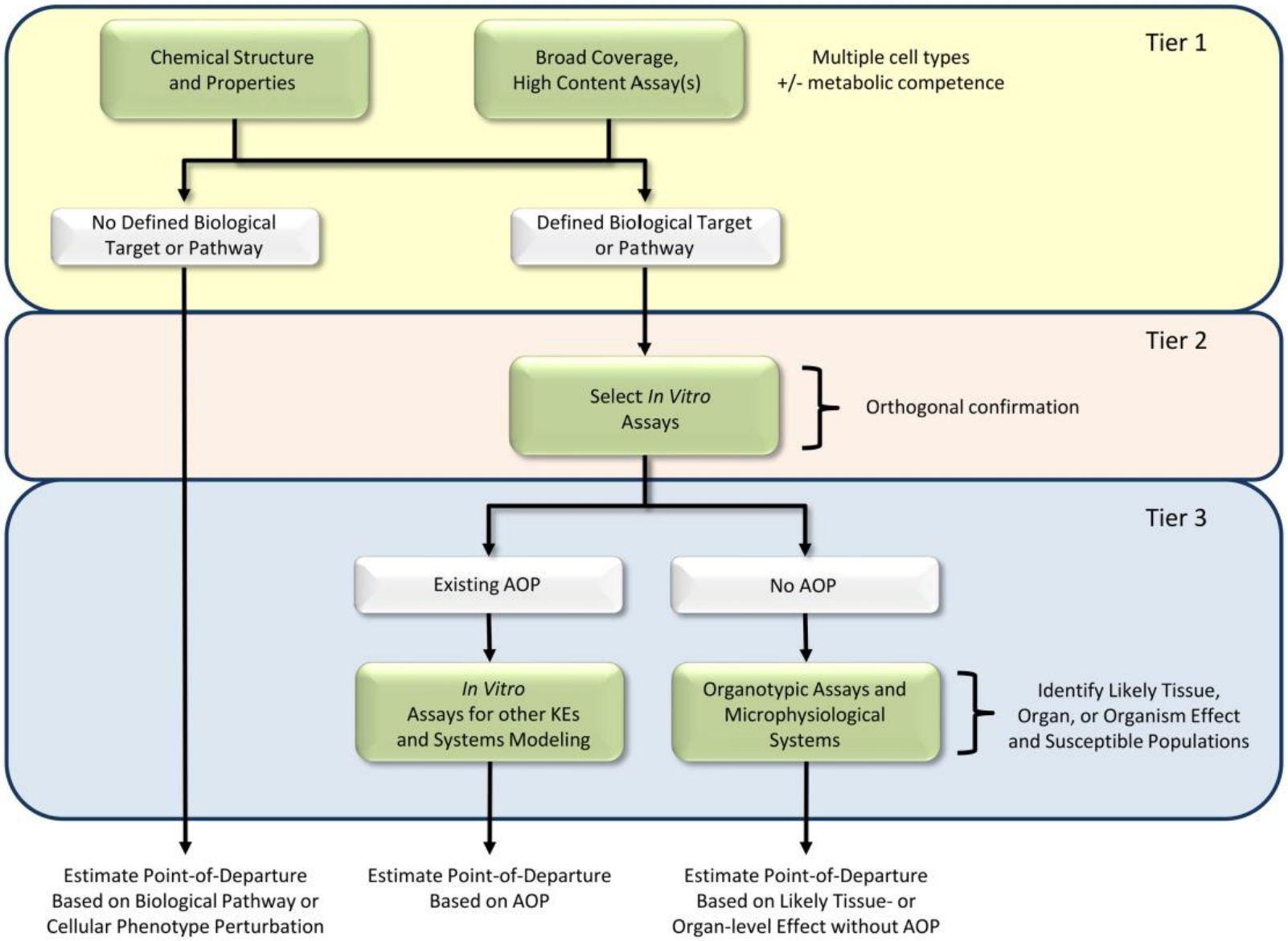

The National Academies of
SCIENCES • ENGINEERING • MEDICINE
REPORT
USING 21ST CENTURY SCIENCE TO IMPROVE RISK-RELATED EVALUATIONS

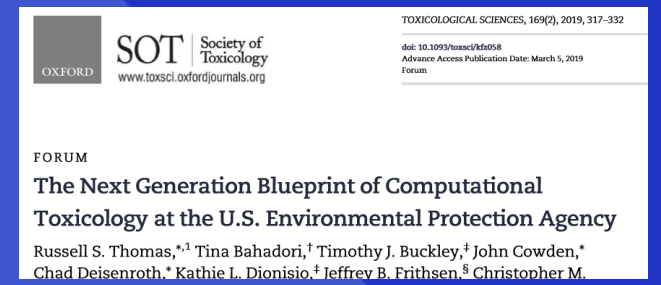

EPA
Next Generation Risk Assessment:
Incorporation of Recent Advances in Molecular, Computational, and Systems
Final Report
National Center for Environmental Assessment
Office of Research and Development


A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States
Utilization
Technology
January 2018
INTERAGENCY COORDINATING COMMITTEE ON THE VALID


U.S. FOOD & DRUG ADMINISTRATION
FDA'S PREDICTIVE
TOXICOLOGY ROADMAP

THE EPA BLUEPRINT




OXFORD SOT Society of Toxicology
www.toxsci.oxfordjournals.org
TOXICOLOGICAL SCIENCES, 169(2), 2019, 317–332
doi: 10.1093/toxsci/kfz058
Advance Access Publication Date: March 5, 2019
Forum

FORUM

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

Russell S. Thomas,*,1 Tina Bahadori,† Timothy J. Buckley,‡ John Cowden,* Chad Deisenroth,* Kathie L. Dionisio,‡ Jeffrey B. Frithsen,§ Christopher M.

Principles of NGRA from ICCR

4 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

3 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

2 Principles for documenting NGRA:

- Sources of uncertainty should be characterized and documented
- The logic of the approach should be transparently and documented

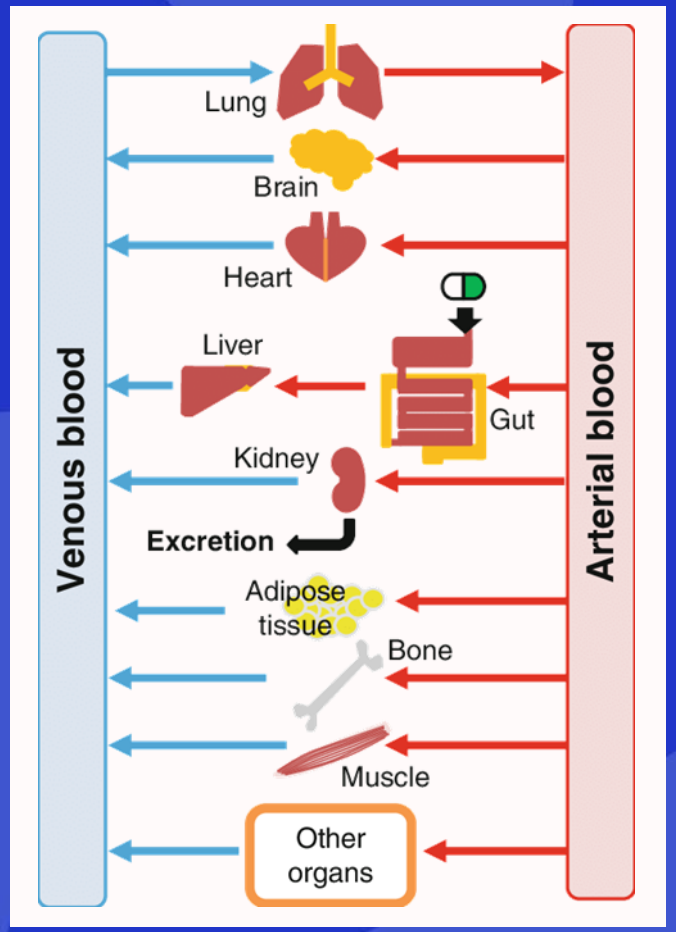
Applied dose

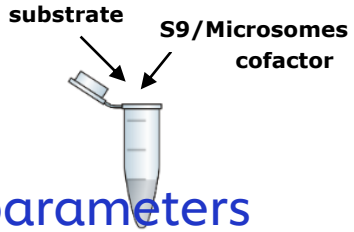


Product types	Face cream	Shampoo	Body Lotion
Amount of product used per day (g/day) using 90th percentile	1.54	10.46	7.82
Frequency of use	2 times/day	1 time/day	2 time/day
Amount of product in contact with skin per occasion (mg)	770	10460	3910
Ingredient inclusion level	0.1%	0.1%	0.1%
Skin surface area (cm2)	565	1440	15670
Leave on or rinse off	leave on	rinse off	leave on
Exposure duration per occasion	12 hours	24 hours	12 hours
For rinse off product, retention factor of finished product on skin [b]	n.a.	0.01	n.a.
Amount of ingredient in contact with skin per occasion (mg)	0.77	0.105	3.91
Local dermal exposure per occasion (µg/cm2)	1.36	0.073	0.25
Systemic exposure per day (mg/kg)	**0.02**	**0.00154**	**0.12**

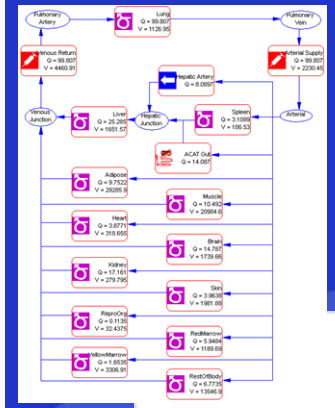
- **Exposures to face cream and body lotion above threshold of toxicological concern (TTC) depending on Cramer classification**
- **Shampoo exposure would be below all non genotoxic TTC**
- **Only face cream and body lotion risk assessment progress to NGRA**

PBK (Physiologically Based Kinetic) Modelling





Model Input:
Physiological parameters
Partition coefficients
Kinetic constants (in vitro)

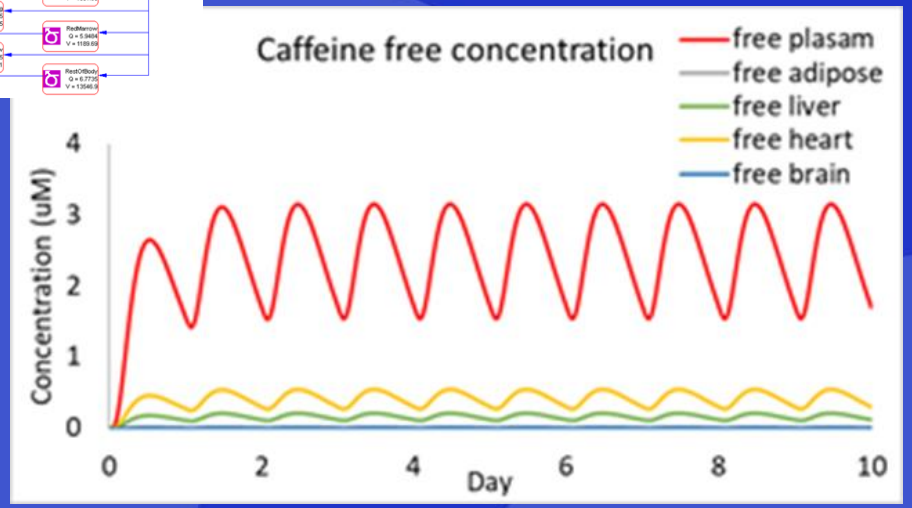


Uptake from GI tract

Transport from arterial to venous blood

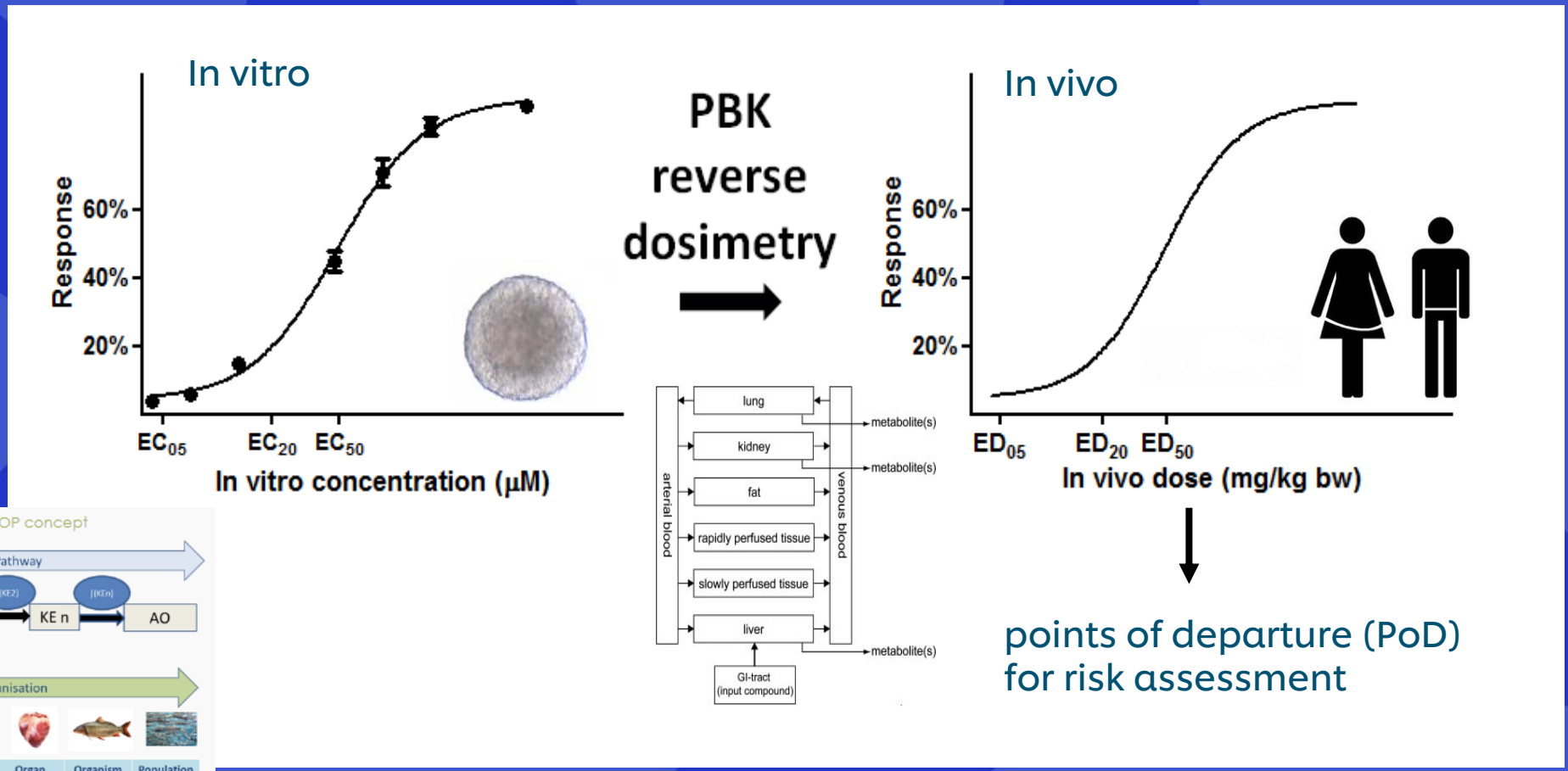
Metabolism

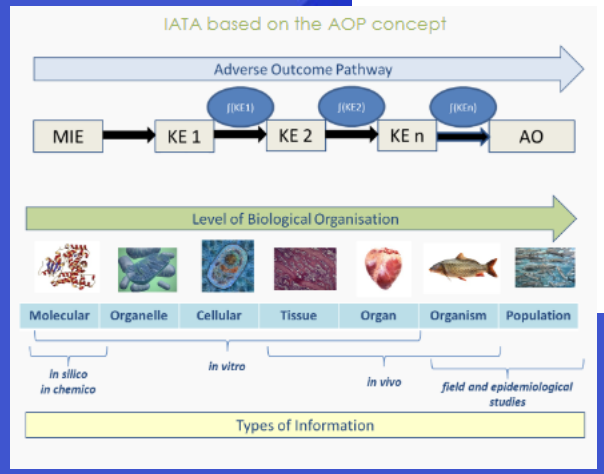
$$dA/dt = + K_A * A_{GI} + QL * (CA - CV) - V_{max} * CL / (K_m + CL)$$



- Predicting systemic exposure
- Enabling us to select and test relevant doses
- Increased role for clinical work to confirm systemic exposure levels

One Interpretation of TT21C: Quantitative *in vitro* to *in vivo* extrapolation

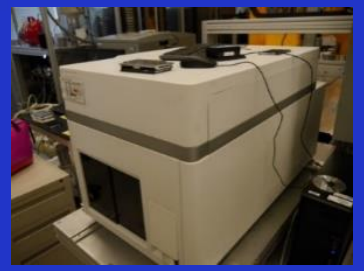




Another Interpretation: Tox21/ToxCast ~700 HTS Biological Pathways Assays






https://www.epa.gov/chemical-research/toxicity-forecasting

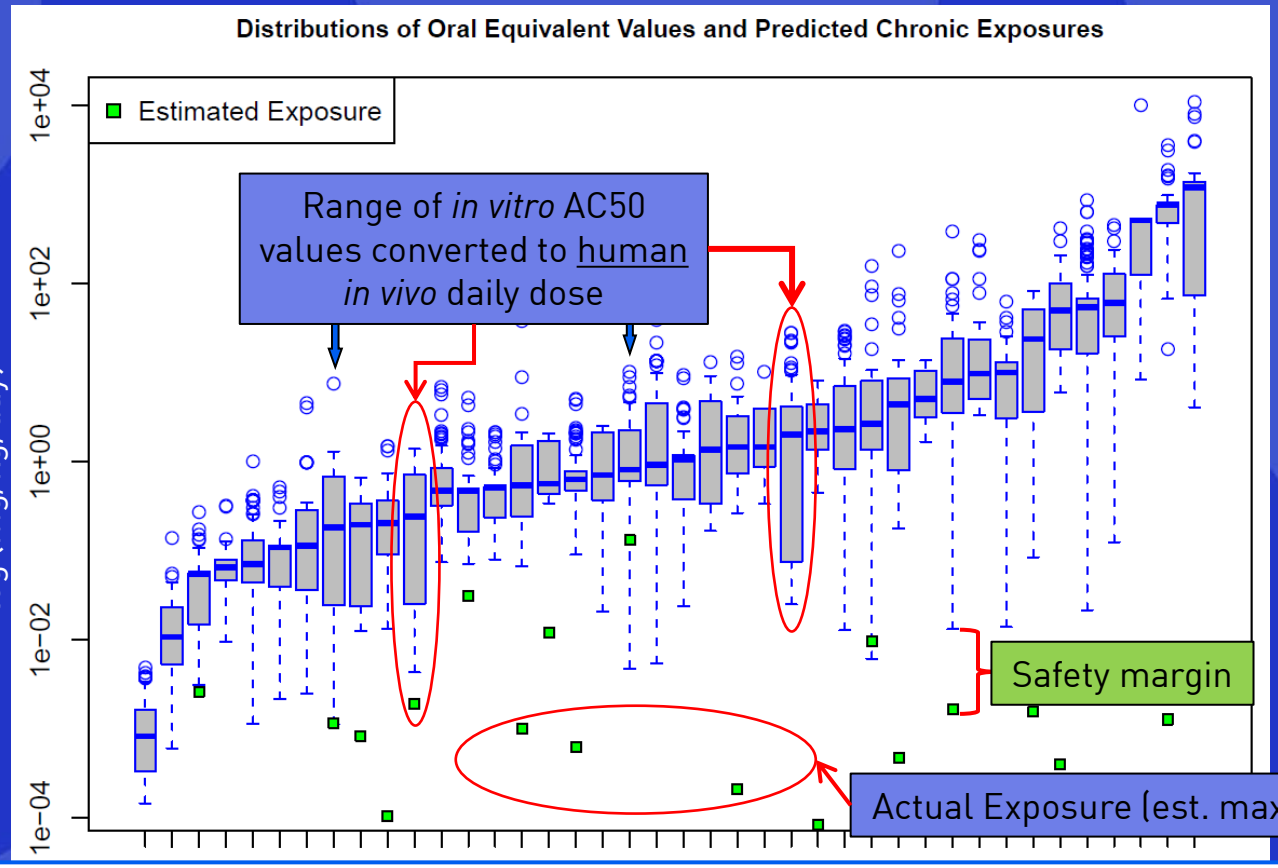
National Institute of Environmental Health Sciences (NIEHS) / National Toxicology Program (NTP)

National Center for Advancing Translational Sciences (NCATS)

U.S. Food and Drug Administration (FDA)

National Center for Computational Toxicology (EPA)

In Vitro Bioactivity vs Bioavailabilty



Hepatic clearance and plasma protein binding determinations

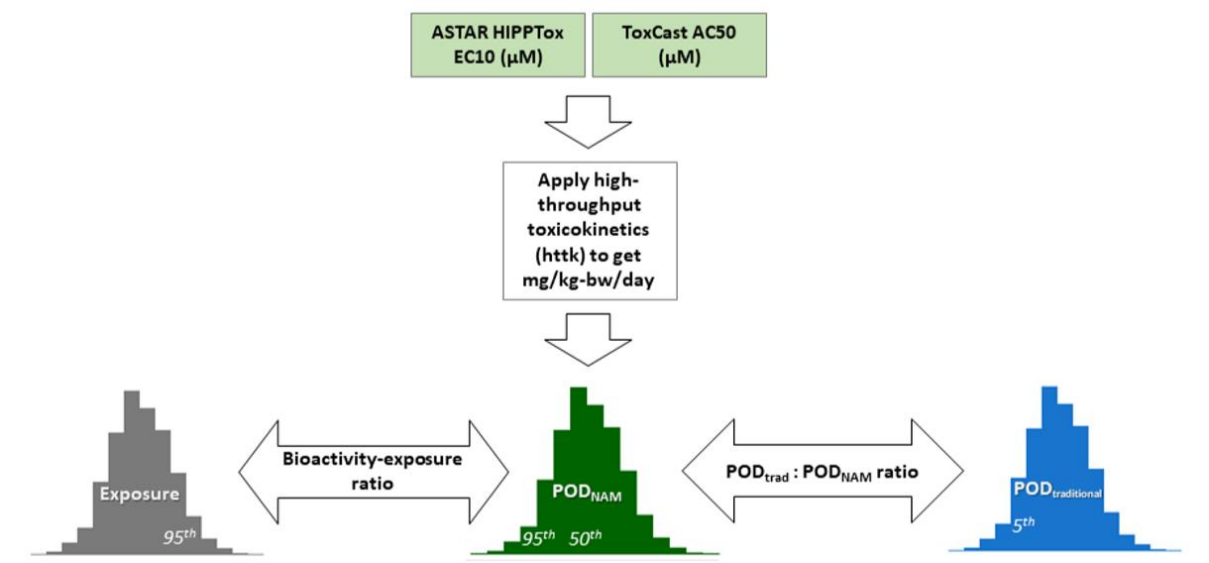
"Protection not Prediction"

Slide from Dr Rusty Thomas, EPA, with thanks

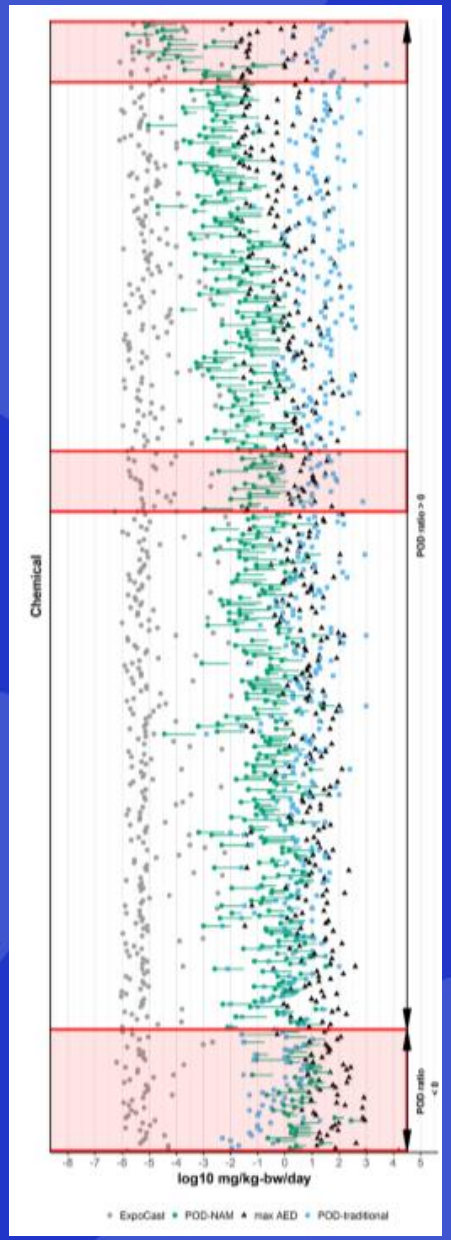
Rotroff, *et al.* Tox.Sci 2010

EPA, NTP, HC, A*STAR, ECHA, EFSA, JRC, RIVM…

APCRA
ACCELERATING THE PACE OF CHEMICAL RISK ASSESSMENT

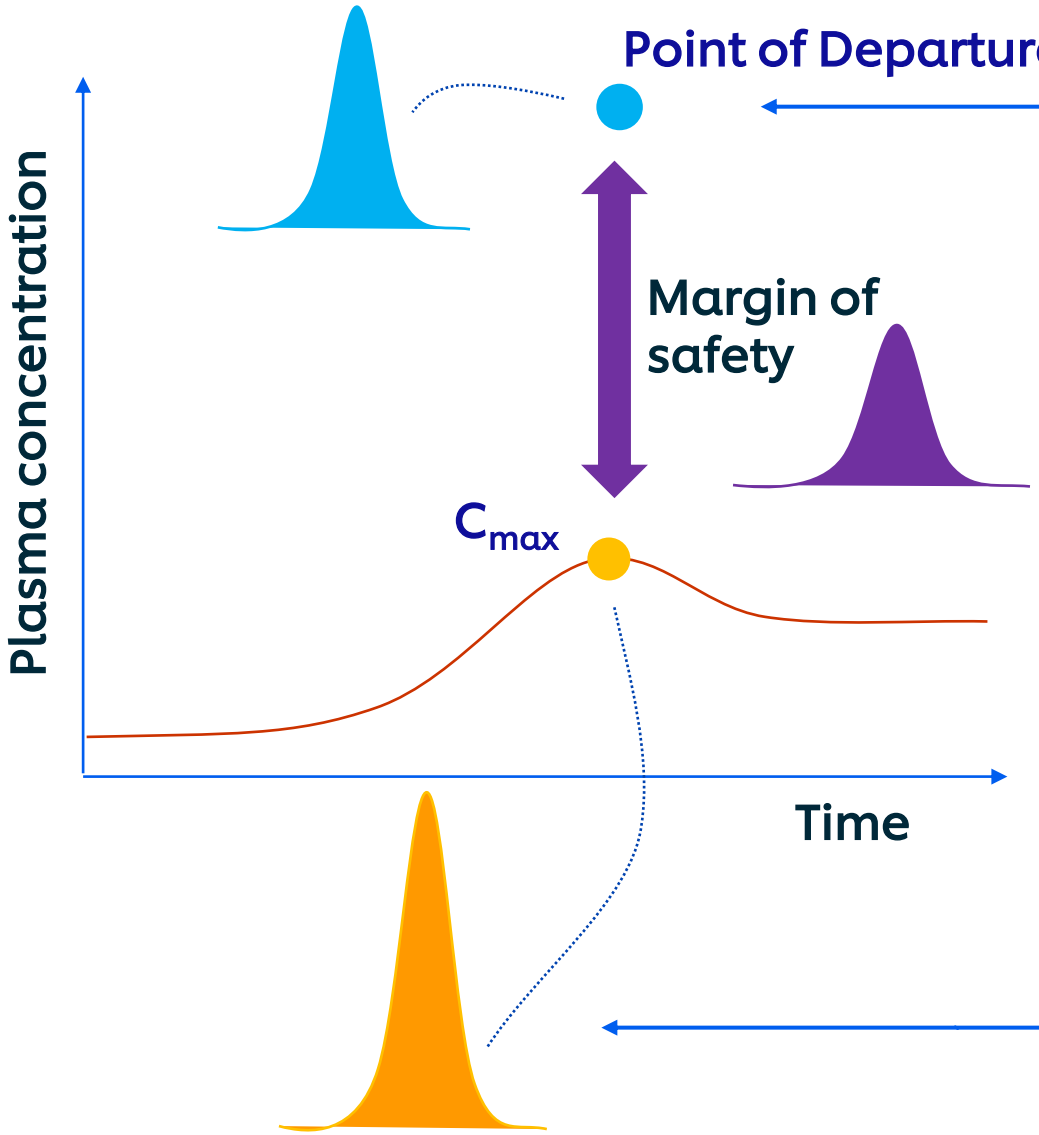


Katie Paul-Friedman *et al.* 2019 Tox Sciences, *October Issue*

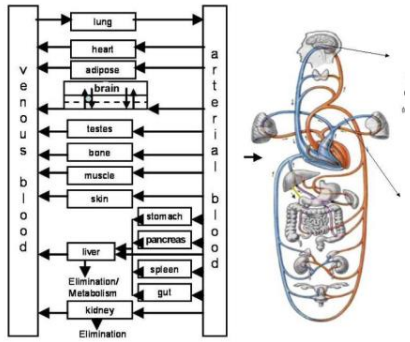


414/448 chemicals =
92% of the time this naïve approach appears conservative

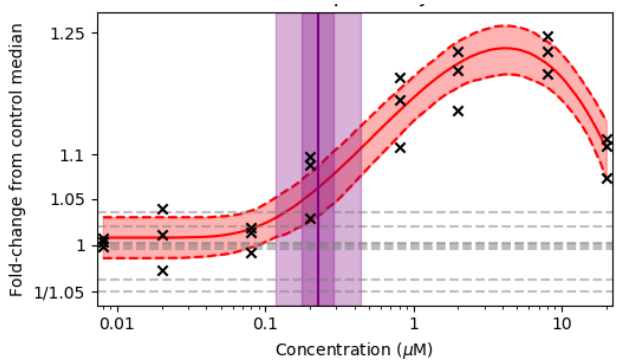
The Margin of Safety Approach



Exposure models (PBK, free/total concentration)



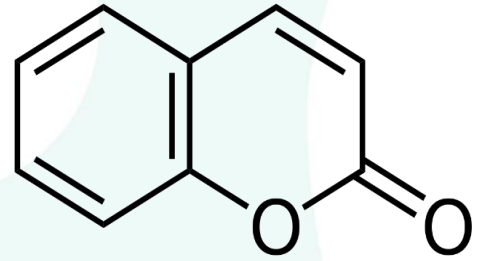
NAM* Point of departure derived from *in vitro* concentration-response



*NAM = New Approach Methodology

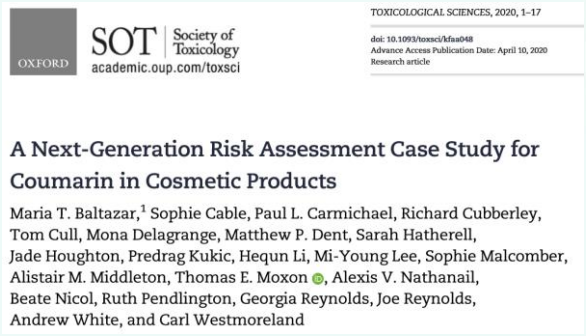
A case study approach – human health safety assessment required for…

0.1% COUMARIN IN FACE CREAM FOR EU MARKET (NEW FRAGRANCE)



Assumed that:

- Coumarin was 100% pure
- No *in vivo* data was available such as animal data, history of safe use (HoSU) or clinical data or use of animal data in read across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academic.oup.com/toxsci

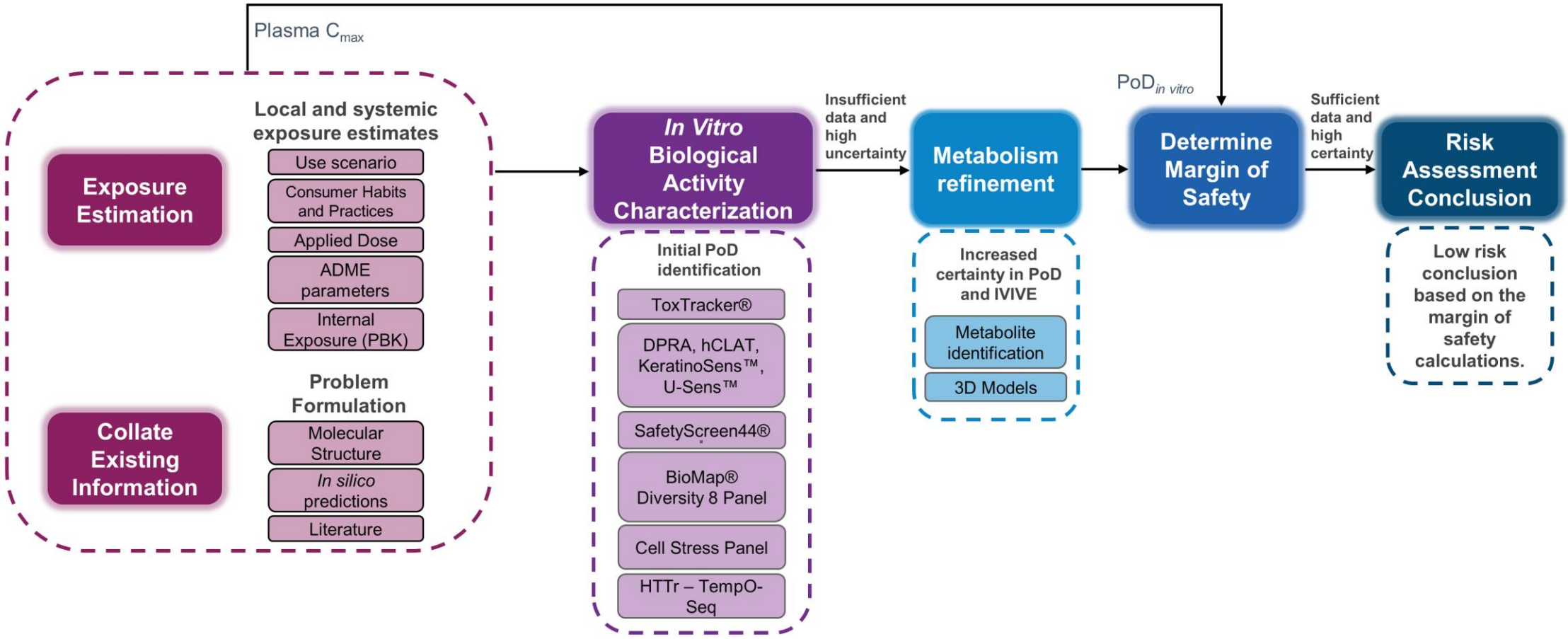
TOXICOLOGICAL SCIENCES, 2020, 1–17
doi: 10.1093/toxsci/kfaa048
Advance Access Publication Date: April 10, 2020
Research article

A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products

Maria T. Baltazar,[1] Sophie Cable, Paul L. Carmichael, Richard Cubberley, Tom Cull, Mona Delagrange, Matthew P. Dent, Sarah Hatherell, Jade Houghton, Predrag Kukic, Hequn Li, Mi-Young Lee, Sophie Malcomber, Alistair M. Middleton, Thomas E. Moxon, Alexis V. Nathanail, Beate Nicol, Ruth Pendlington, Georgia Reynolds, Joe Reynolds, Andrew White, and Carl Westmoreland

Baltazar *et al.*, (2020) *Tox Sci* Volume 176, Issue 1, 236–252

Next-Generation Risk Assessment case study workflow for 0.1% coumarin in face cream



Baltazar *et al.*, (2020) *Tox Sci* Volume 176, Issue 1, 236–252

NAMs used to estimate internal concentration

Exposure Estimation

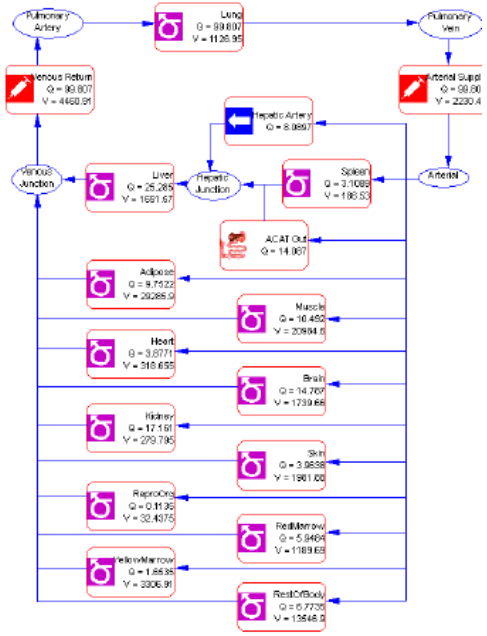
Local and systemic exposure estimates

- Use scenario
- Consumer Habits and Practices
- Applied Dose
- ADME parameters
- Internal Exposure (PBK)

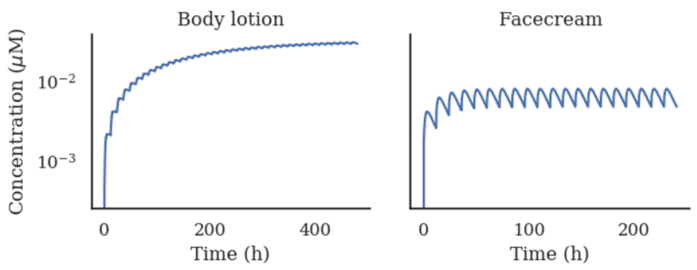
ADME & Physico-Chemical parameters to generate

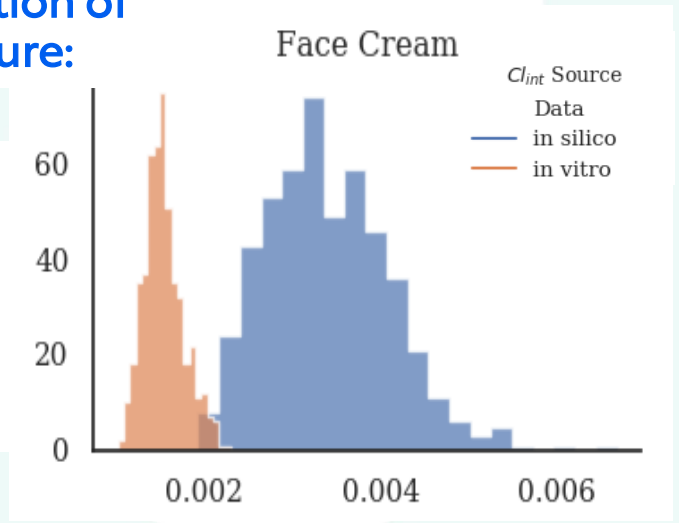
- logP, f_{up}, R_{bp} (1.39, 0.3, 0.7)
- Hepatic Clearance
  - Hepatocyte only (929 L/h)
- CYP Stability
  - Stable in all but **CYP2A6**
- Skin Penetration

GastroPlus® (Simulations Plus)



Simulated plasma concentration of coumarin after dermal exposure:





Moxon *et al.*, (2020). Application of physiologically based kinetic (PBK) modelling in the next generation risk assessment of dermally applied consumer products. Toxicology in Vitro Volume 63

Unilever

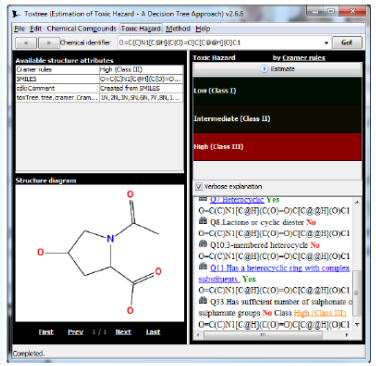
NAMs used to predict biological activity based on chemical structure

Collate Existing Information

Problem Formulation

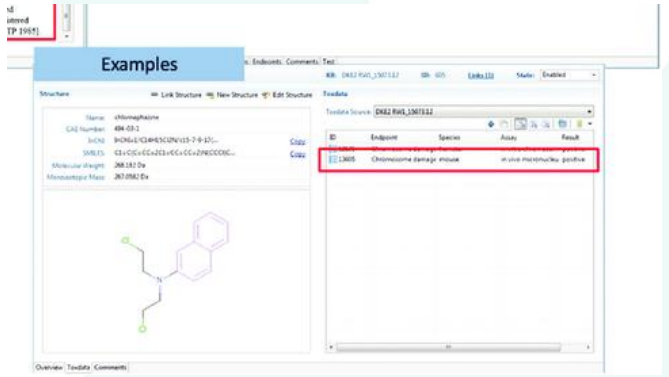
- Molecular Structure
- *In silico* predictions
- Literature

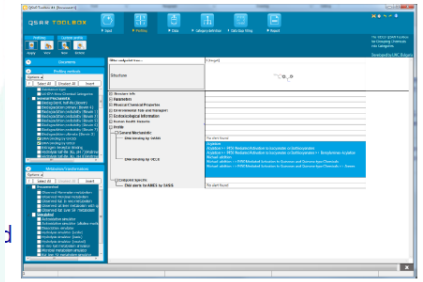
ToxTree



Derek nexus

OECD QSAR TOOLBOX





In silico models to predict Molecular initiating events (MIEs)

OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org ToxSci 20 Years

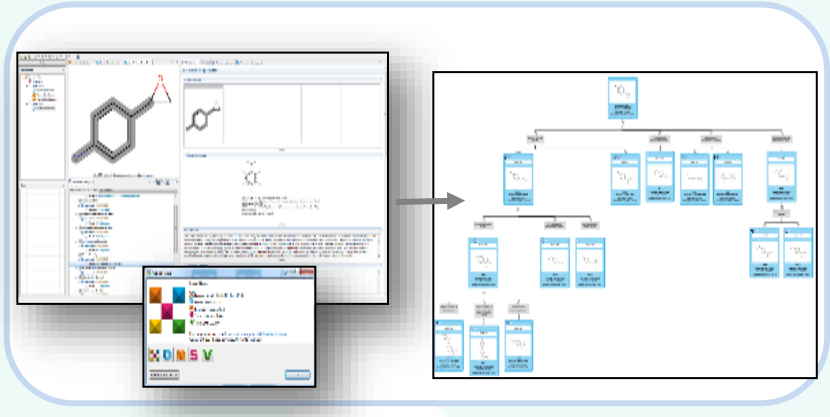
TOXICOLOGICAL SCIENCES, 165(1), 2018, 213–223

doi: 10.1093/toxsci/kfy144
Advance Access Publication Date: July 18, 2018
Research Article

Using 2D Structural Alerts to Define Chemical Categories for Molecular Initiating Events

Timothy E. H. Allen,* Jonathan M. Goodman,*,1 Steve Gutsell,† and Paul J. Russell†

Meteor nexus



Unilever

NAMs used to characterize the biological activity of coumarin

***In Vitro* Biological Activity Characterization**

Initial PoD identification

- ToxTracker®
- SafetyScreen44®
- BioMap® Diversity 8 Panel
- Cell Stress Panel
- HTTr – TempO-Seq

To investigate possible interactions between coumarin and the 83 key targets involved in drug attrition

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 earlier in the drug discovery process to identify undesirable off-target activity profiles that could hinder or halt the development of candidate drugs or even lead to market withdrawal if discovered after a drug is approved. Here, for the first time, the rationale, strategies and methodologies for *in vitro* pharmacological profiling at four major pharmaceutical companies (AstraZeneca, GlaxoSmithKline, Novartis and Pfizer) are presented and illustrated with examples of their impact on the drug discovery process. We hope that this will enable other companies and academic institutions to benefit from this knowledge and consider joining us in 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.

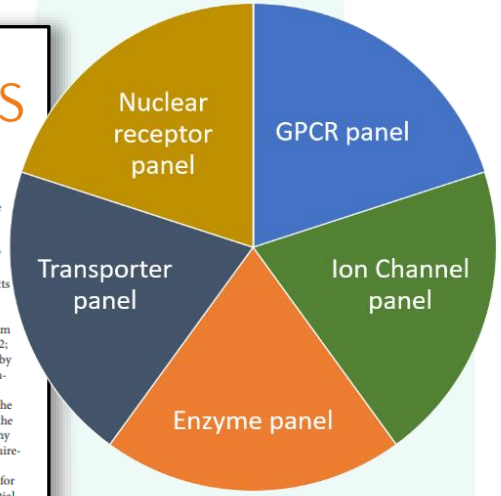
In vitro pharmacological profiling involves the screening of compounds against a broad range of targets (receptors, ion channels, enzymes and transporters) that are distinct from the intended

safety testing of drug candidates and are designed to prevent serious ADRs from occurring in clinical studies.

The only *in vitro* pharmacology assay that is absolutely required by regulatory authorities is one that measures the effects of new chemical entities on the ionic current of native (I_{Kr}) or heterologously expressed human voltage-gated potassium channel subfamily H member 2 (KCNH2; also known as hERG)[2]. The mechanism by which blockade of hERG can elicit potentially fatal cardiac arrhythmias (torsades de pointes) following a prolongation of the QT interval is well characterized[2,3], and the seriousness of this ADR is one reason why this assay is a mandatory regulatory requirement. Receptor binding studies are also recommended as the first-tier approach for the assessment of the dependence potential of novel chemical entities[3].

However, current regulatory guidance does not describe which targets should constitute an *in vitro* pharmacological profiling panel and does not indicate the stage of the discovery process at which *in vitro* pharmacological profiling should occur. Nevertheless, the general trend for most pharmaceutical companies is to perform this testing early in drug discovery to reduce attrition and to facilitate better prediction of ADRs in the later stages of drug discovery and development.

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



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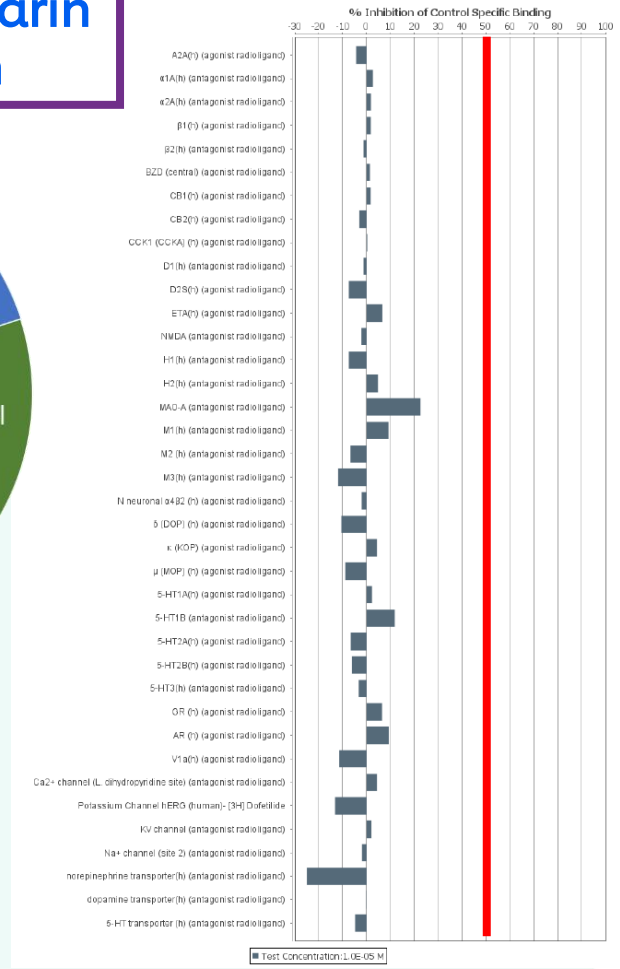

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NAMs used to characterize the biological activity of coumarin

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In Vitro Biological Activity Characterization

Initial PoD identification

- ToxTracker®
- SafetyScreen44®
- BioMap® Diversity 8 Panel
- Cell Stress Panel
- HTTr – TempO-Seq

36 Biomarkers;
3 Timepoints;
8 Concentrations;
~10 Stress Pathways

- Mitochondrial Toxicity
- Oxidative Damage
- DNA damage
- Inflammation
- ER stress
- Metal stress
- Heat Shock
- Hypoxia
- Cell Health

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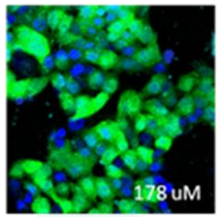
TOXICOLOGICAL SCIENCES, 2020, 1–23
doi: 10.1093/toxsci/kfaa054
Advance Access Publication Date: May 6, 2020
Research article

FEATURED

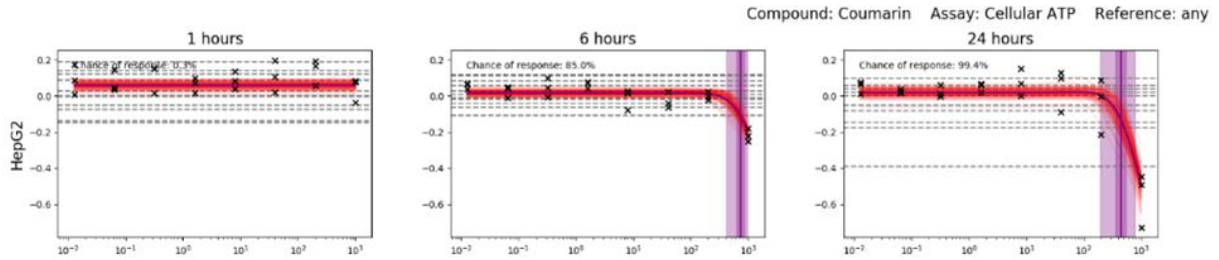
Identifying and Characterizing Stress Pathways of Concern for Consumer Safety in Next-Generation Risk Assessment

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

*Unilever Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, Bedfordshire



Dose-response analysis and in vitro PoD derivation



Biomarkers	Cell type	Stress pathway	PoD (µM)	Effect	Concentration dependency score (CDS)
ATP (6h)	HepG2	cell health	794 (363-977)	down	0.98
ATP (24h)			617 (282-891)	down	1
Phospholipidosis (24h)	HepG2	cell health	759 (437-977)	down	0.93
GSH (24h)	HepG2	oxidative stress	851 (301-1000)	up	0.92
IL-8 (24h)	HepG2	inflammation	912 (575-1000)	down	0.61
OCR (1h)	NHEK	mitochondrial toxicity	62 (2.6-776)	down	0.6
OCR (6h)			468 (214-794)		1
OCR (24h)			309 (138-1000)		0.52
Reserve capacity (1h)	NHEK	mitochondrial toxicity	44 (23-96)	down	1
Reserve capacity (6h)			759 (302-1000)		0.9
Reserve capacity (24h)			794 (295-1000)		0.55

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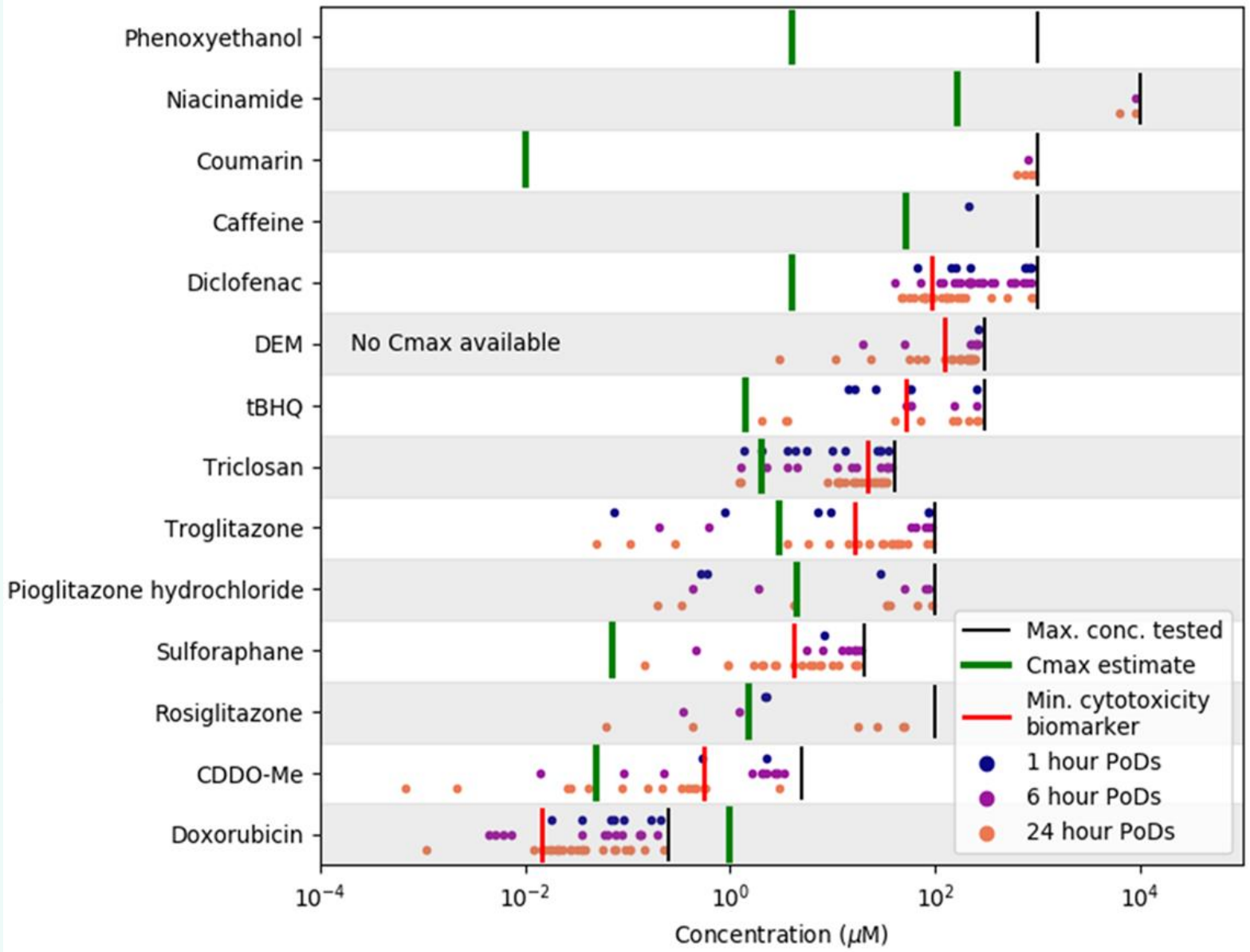

Phenoxyethanol
Niacinamide
Coumarin
Caffeine
Diclofenac
DEM
No Cmax available
tBHQ
Triclosan
Troglitazone
Pioglitazone hydrochloride
Sulforaphane
Rosiglitazone
CDDO-Me
Doxorubicin
Max. conc. tested
Cmax estimate
Min. cytotoxicity biomarker
1 hour PoDs
6 hour PoDs
24 hour PoDs
10^{-4}
10^{-2}
10^{0}
10^{2}
10^{4}
Concentration (μM)

NAMs for in vitro bioactivity: HTTr (Tempo-Seq)

High-Throughput Transcriptomics Gene Expression Profiling (HTTr)

1. Defining a safe operating exposure for systemic toxicity using a NOTEL (No Transcriptional Effect Level)
2. Defining compound similarity grouping (Read Across)

NOTEL is the derived concentration of a compound that does not elicit a meaningful change in gene expression (i.e. the threshold of the concentration that elicits minimal mechanistic activity)

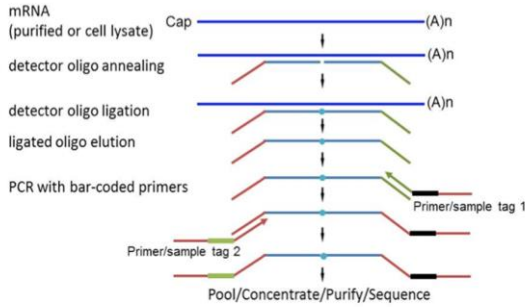
Cell lines (chosen to express a range of relevant receptors)

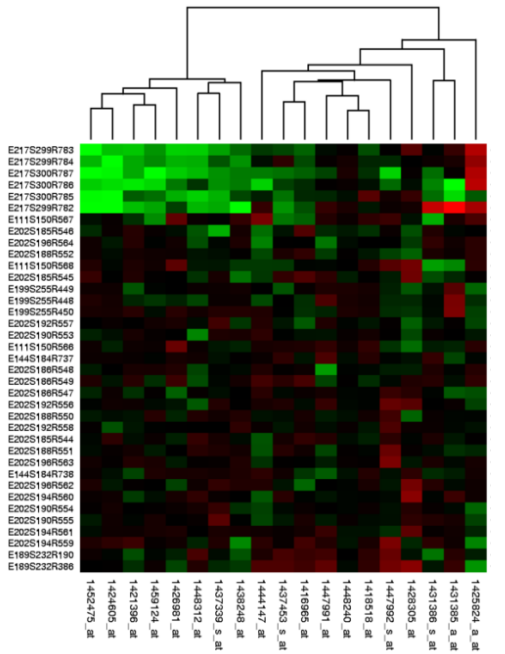
MCF-7 – human breast adenocarcinoma cell line

HepG2 – human liver carcinoma

HepaRG – terminally differentiated hepatic cells that retain many characteristics of primary human hepatocytes + as spheroids

N-HEK – primary normal human epidermal keratinocytes





NAMS used to characterize the biological activity of coumarin

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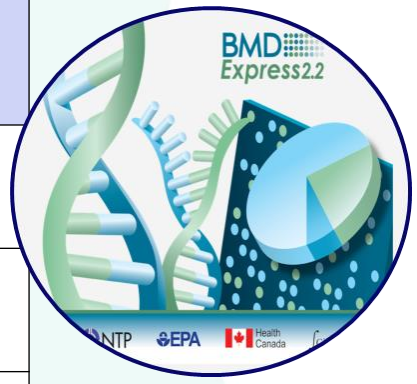
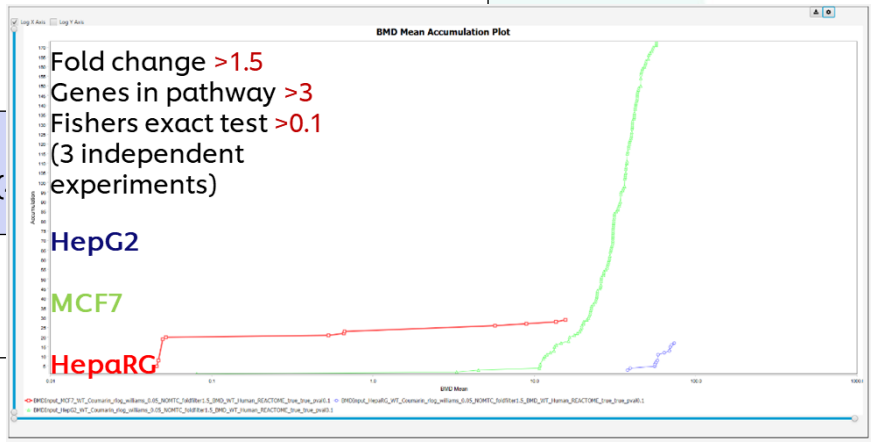
- SafetyScreen44®
- BioMap® Diversity 8 Panel
- Cell Stress Panel
- HTTr – TempO-Seq

Transcriptomics can be applied as a broad nontargeted biological screen – PoD determination using BMDexpress

Cell model	HepG2	MCF7	HepaRG 2D
Pathway level tests PoD_T (µM)	(308 pathways)	(0 pathways)	(17 pathways)
20 pathways with the lowest p value Reactome	70	NA	58*
20 pathways with the lowest BMD Reactome	44	NA	58*
BMD of Reactome pathway with lowest BMD that meets significance threshold criteria	31		
Gene level tests PoD_T (µM)	(1570 genes)	(	
Mean BMD of 20 genes with largest fold change	6		
Mean BMD of genes between 25th and 75th percentile	17		





Farmahin, R., Williams, A., Kuo, B. *et al.* Recommended approaches in the application of toxicogenomics to derive points of departure for chemical risk assessment. *Arch Toxicol* **91,** 2045–2065 (2017). https://doi.org/10.1007/s00204-016-1886-5

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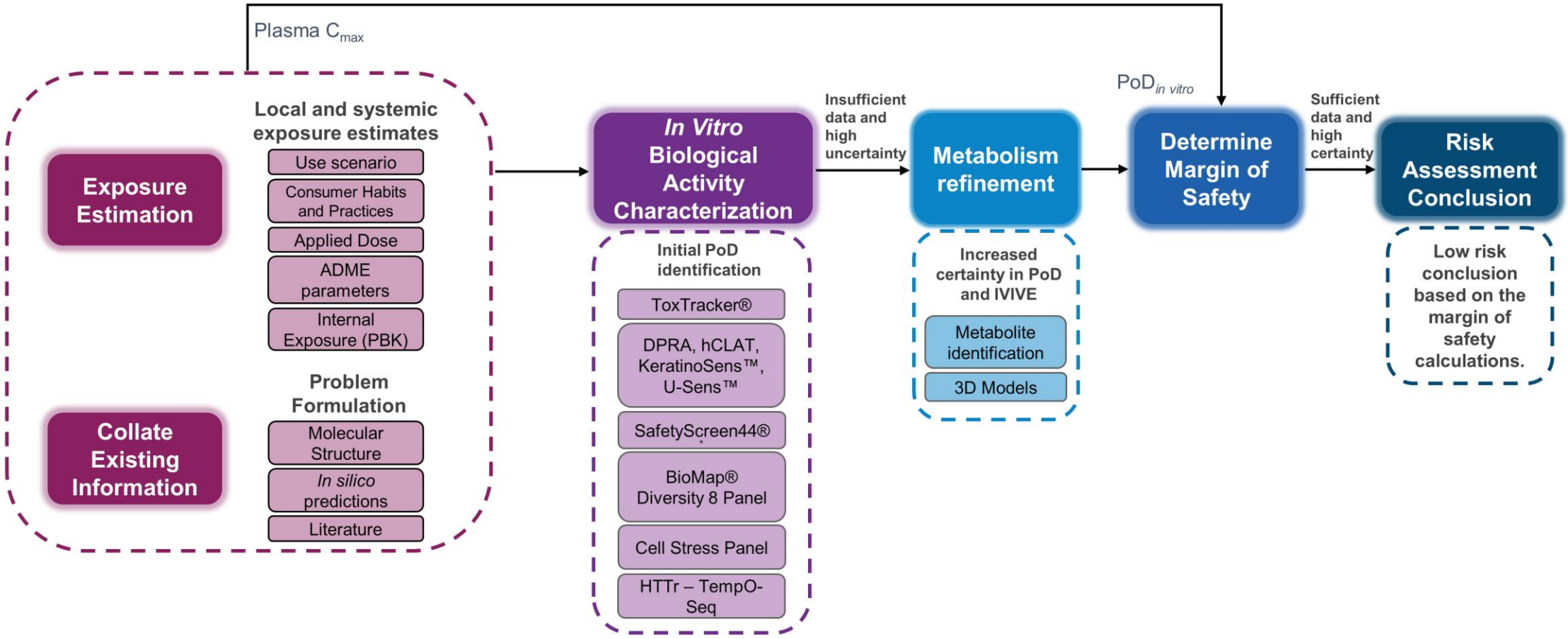
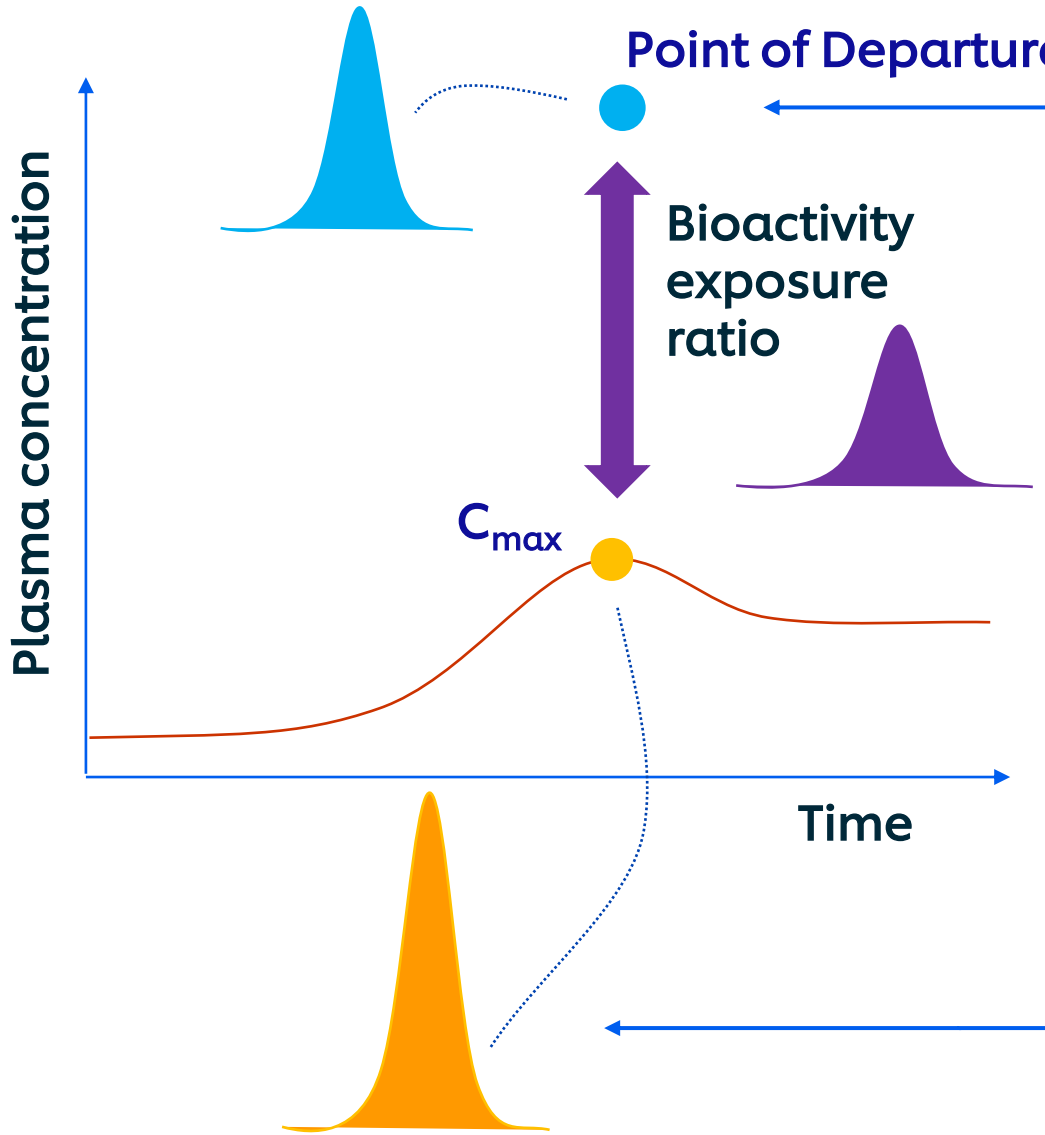
Next-Generation Risk Assessment case study workflow for 0.1% coumarin in face cream

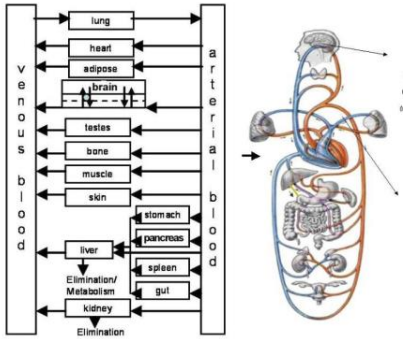


Baltazar *et al.*, (2020) *Tox Sci* Volume 176, Issue 1, 236–252

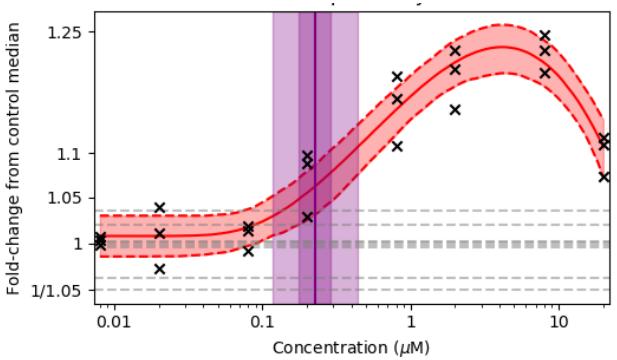
The Margin of Safety Approach



Exposure models (PBK, free/total concentration)



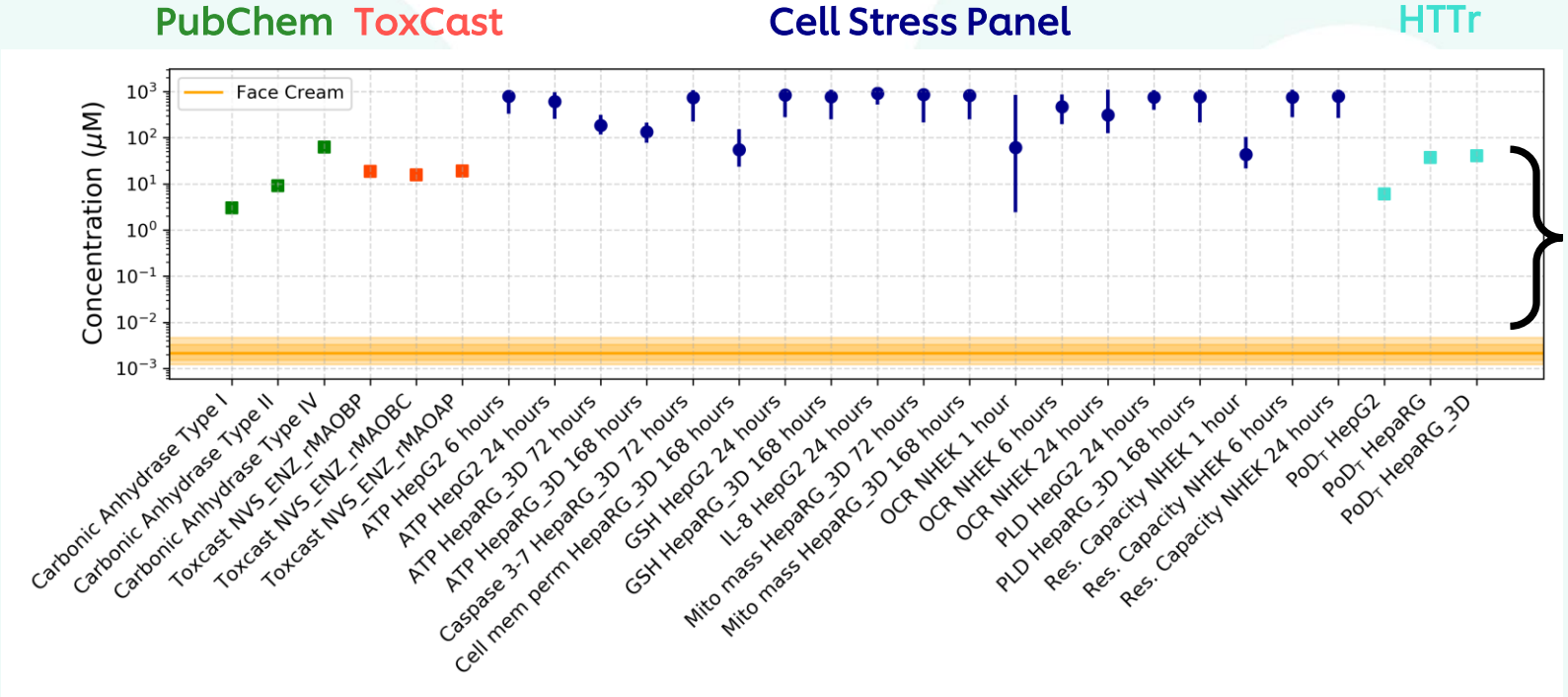
NAM* Point of departure derived from *in vitro* concentration-response



*NAM = New Approach Methodology

Determination of MoS using NAMs and risk assessment conclusion

Determine Margin of Safety



Margin of safety

The 5th percentile of the MoS distribution ranged between 706 and 96738

In this case study:

- **Weight of evidence suggested that the inclusion of 0.1% coumarin in face cream is safe for the consumer**

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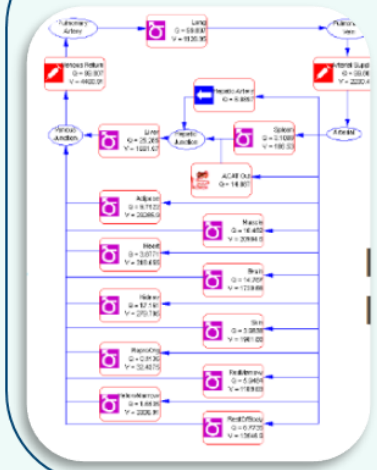
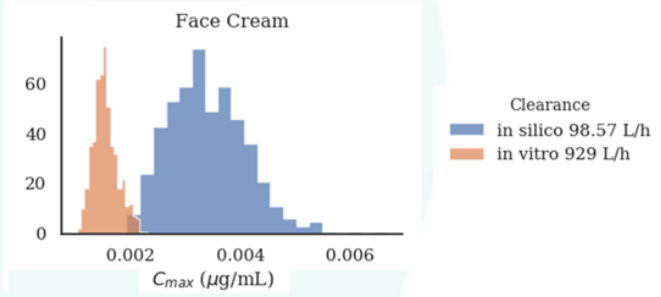
The Key Elements in our NGRA Approach

PBK Modelling





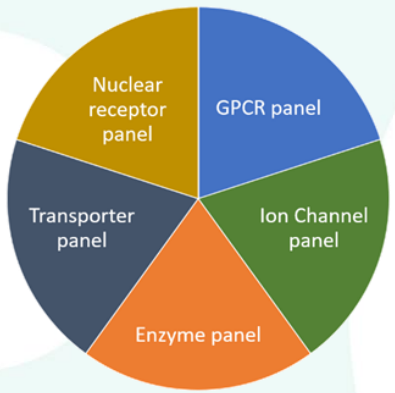
Toxicology in Vitro (2020), **63**, 104746

In vitro pharmacological profiling


PERSPECTIVES

A GUIDE TO DRUG DISCOVERY — OPINION

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



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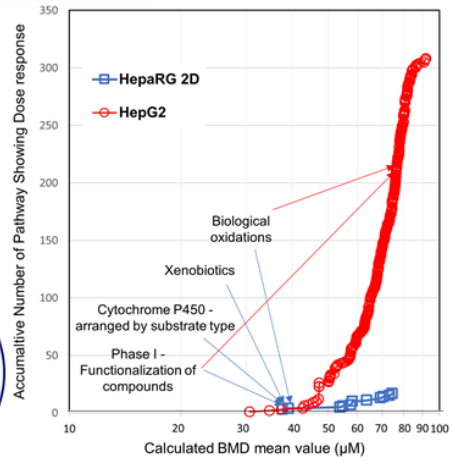
Transcriptomics

- Use of full human gene panel ~ 21k
- 24 hrs exposure
- 7 concentrations
- 3 cell lines HepG2/ HepaRG/ MCF7
- 3D HepaRG spheroid

BMDExpress 2



Cellular Stress Pathways

13 chemicals, 36 Biomarkers; 3 Timepoints; 8 Concentrations; ~10 Stress Pathways

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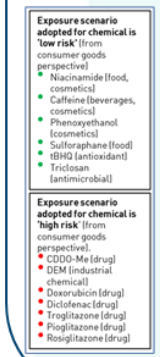

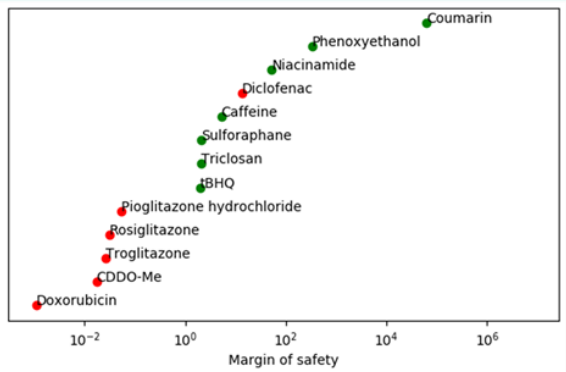

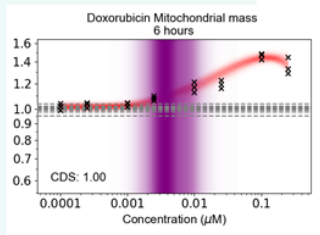

Toxicol Sci (2020), **176**, 11-33

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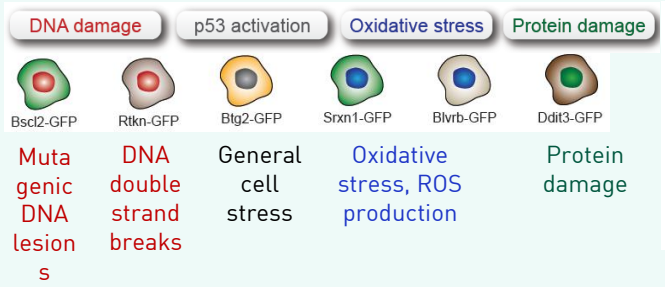
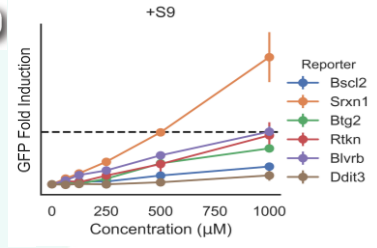
NGRA is hypothesis-driven – examples of bespoke assays used in the coumarin case study

Genotoxicity assessment: ToxTracker®

- Coumarin and its metabolites triggered genotoxicity alerts

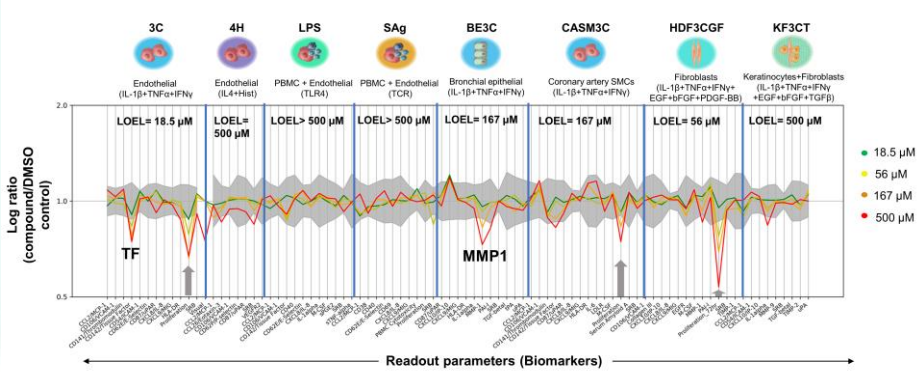




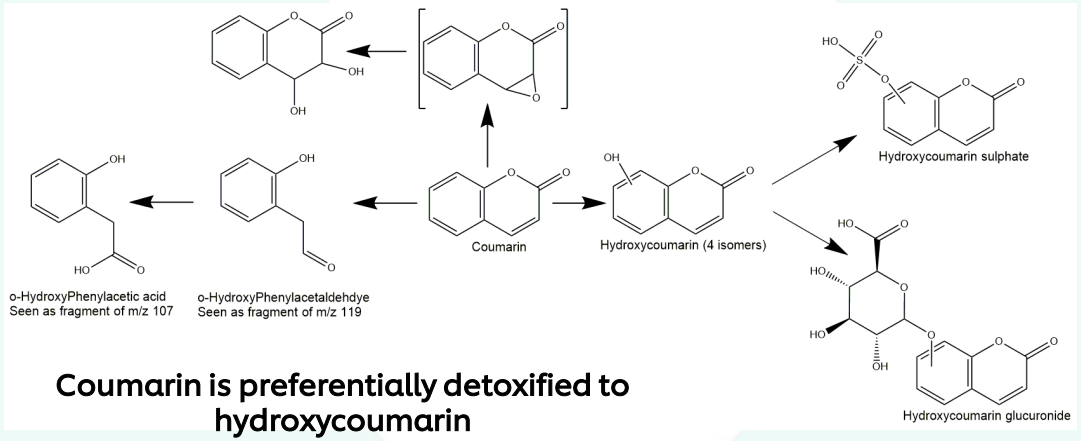
6 GFP reporter mouse embryonic stem (mES) cells

Immunomodulatory screening assay: BioMap® Diversity 8 Panel

- Coumarin predicted to have anti-inflammatory properties



Metabolite identification & PoD refinement

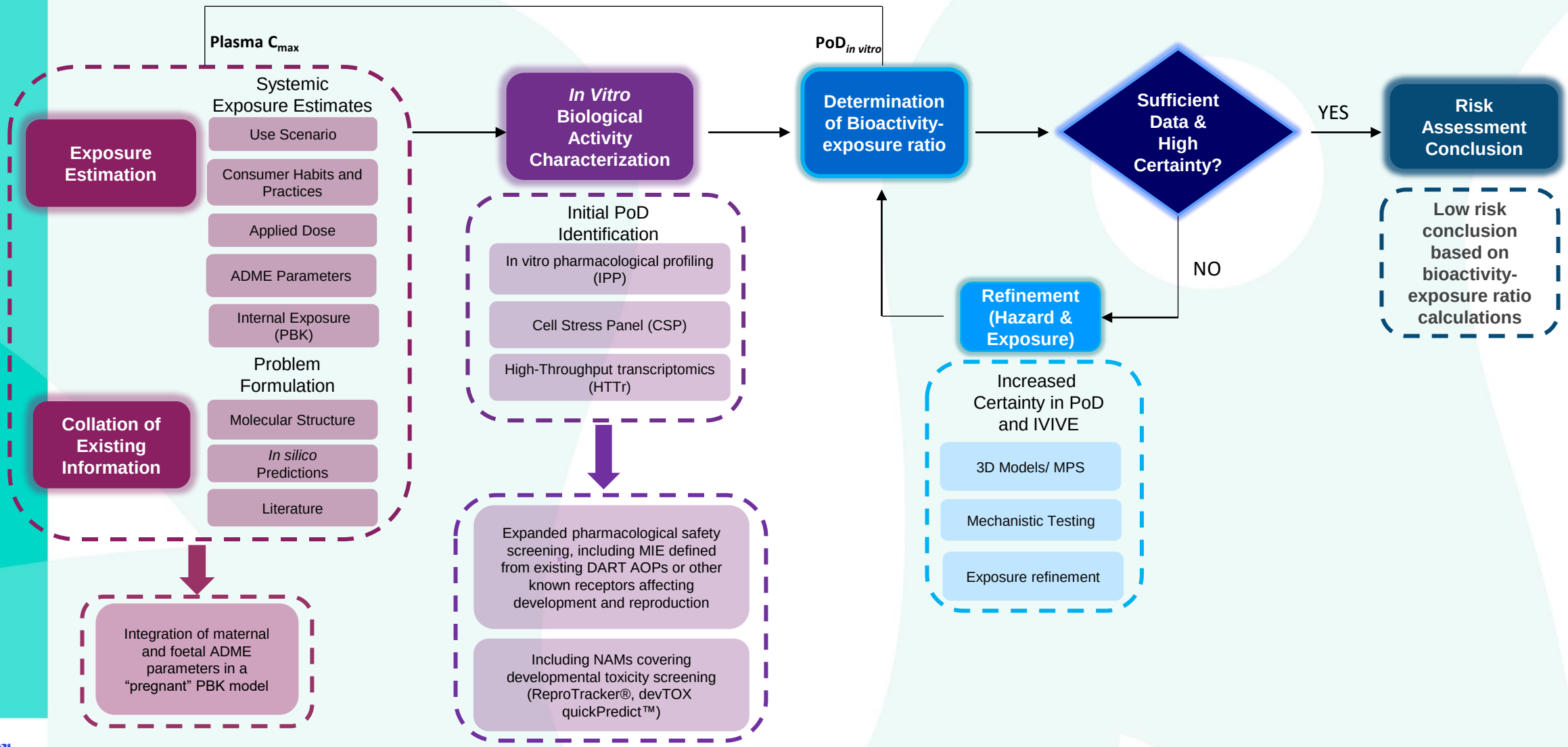


Coumarin is preferentially detoxified to hydroxycoumarin

Cell stress & HTTr in 3D HepaRG models

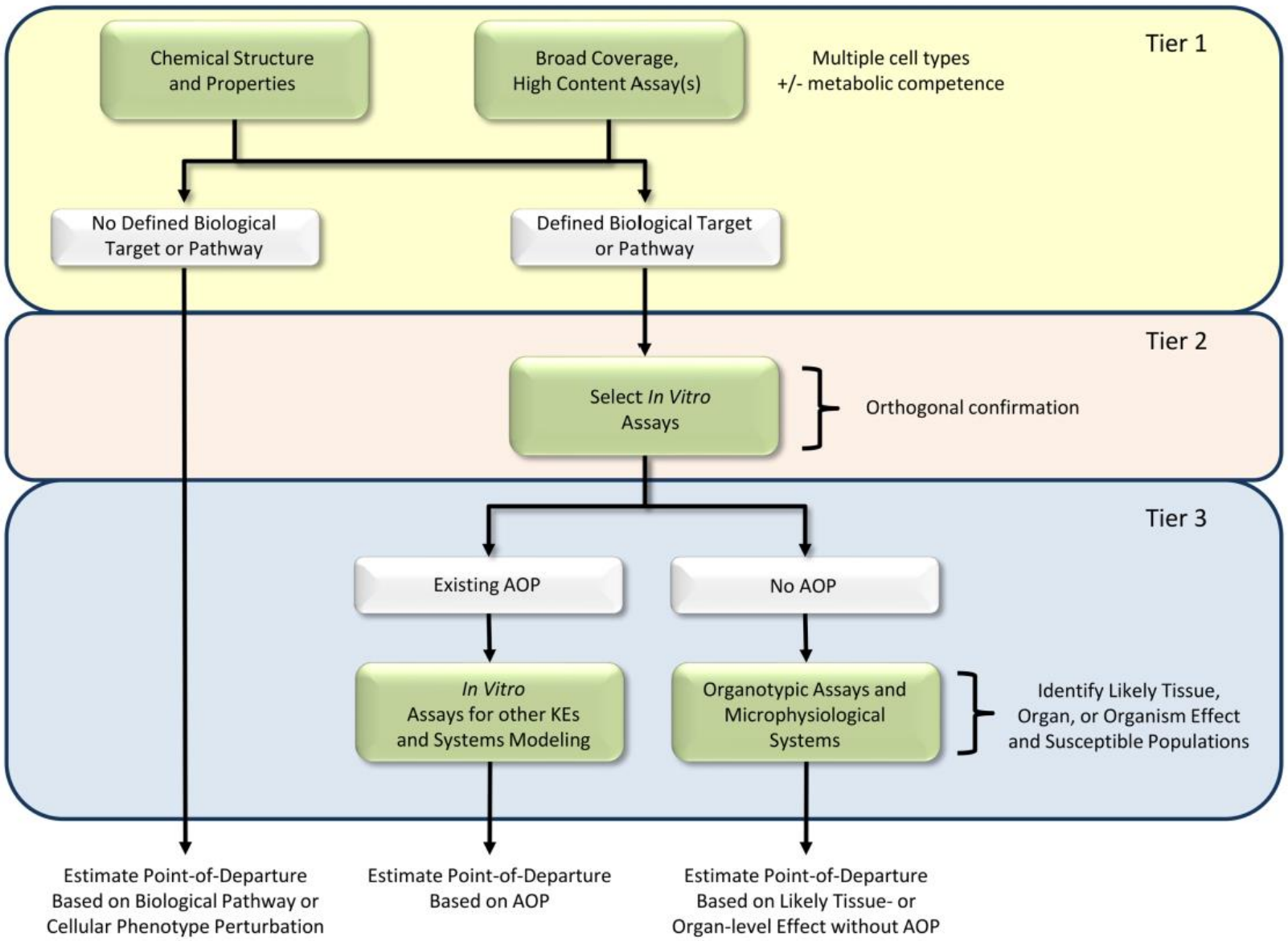
- Low bioactivity also found in a metabolic competent cell model (HepaRG 3D)
- PoDs range: 41-871 µM – similar range as in from 2D cells

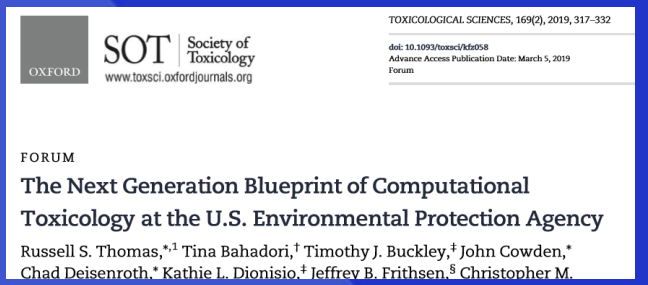
Integrating DART Safety Assessment into Existing NGRA Framework



An NGRA framework with additional NAMs relevant for DART endpoints

The EPA Blueprint

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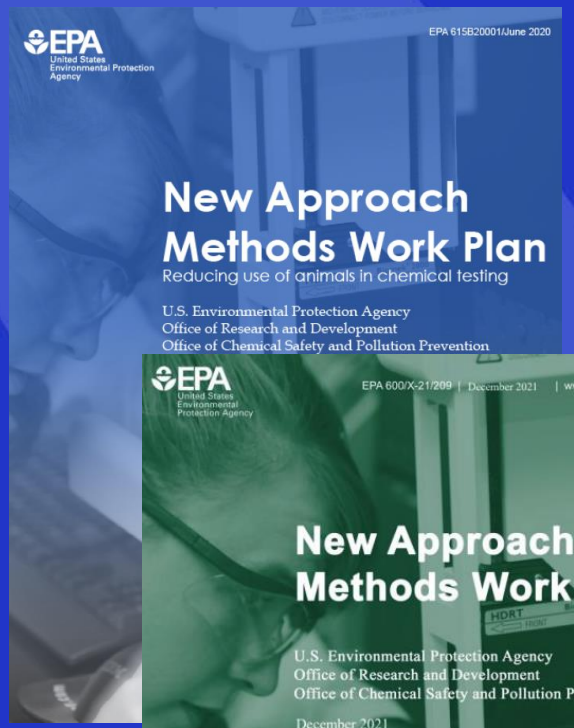
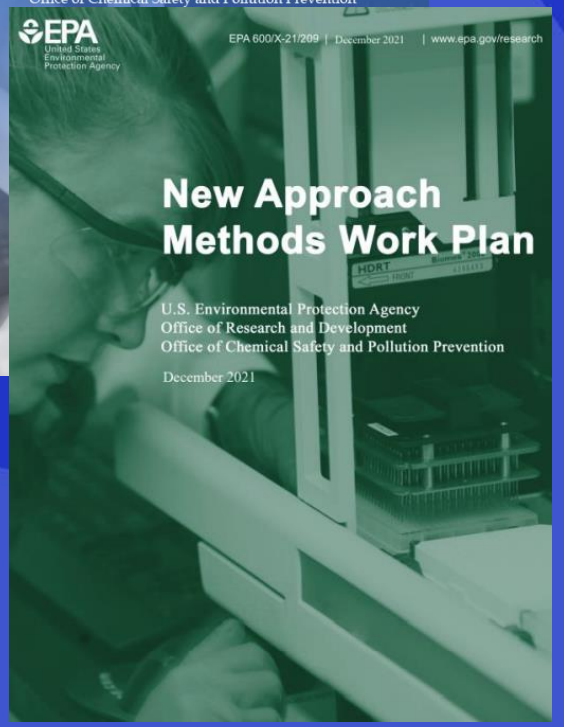
doi: 10.1093/toxsci/kfz058
Advance Access Publication Date: March 5, 2019
Forum

FORUM

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

Russell S. Thomas,*,[1] Tina Bahadori,[†] Timothy J. Buckley,[‡] John Cowden,* Chad Deisenroth,* Kathie L. Dionisio,[‡] Jeffrey B. Frithsen,[§] Christopher M.

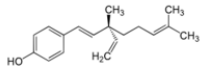




Dent *et al.*, (2018) Toxicological Sciences

Androgen Receptor Antagonism

- Problem formulation: Can Bakuchiol be safely used at 0.5% in a body lotion or a shampoo?
  - Calculate exposure –above TTC for both exposure scenarios
  - Perform literature search – no 'definitive' toxicology data but indications of hormonal activity
  - In-silico screen – suggestive of AR interaction



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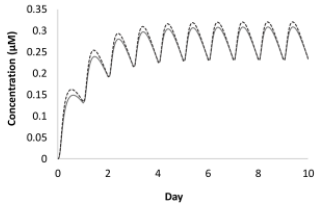
Physiologically-Based Kinetic Modelling

- Low-tier assessment based on predicted/scaled values



Predicted concentration in plasma for females (dotted line) and males (solid line) following daily use of a body lotion containing Bakuchiol at 0.5%

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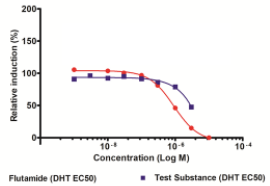
Bakuchiol Dose Response Data

- Dose-response data generated in a human-relevant system
- (AR-CALUX® assay)



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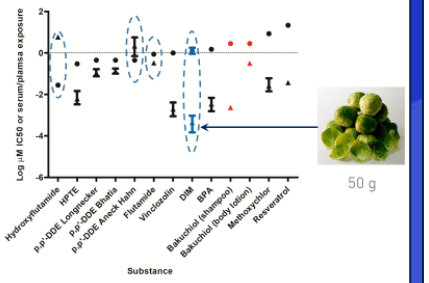
Comparing Exposure and Effect Concentrations

Triangles show plasma or serum levels, circles show IC_{50} values for bakuchiol and several anti-androgens



50 g

What is an appropriate 'Margin of Exposure'?

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Using Dietary Comparator Ratios to Benchmark Risk

- Calculation of Exposure:Activity Ratios (After Becker *et al.* 2015 *Regul. Toxicol. Pharmacol.* 71(3), 398–408):

$$\text{EAR (unitless)} = \frac{\text{Exposure (plasma exposure in µM)}}{\text{Activity } (IC_{50}\ \mu M)}$$

$$\text{DCR} = \frac{\text{EAR (test substance)}}{\text{EAR (dietary comparator)}}$$

If DCR<1 the activity of the test substance exposure would be lower than the activity of the dietary comparator exposure which has a history of safe use

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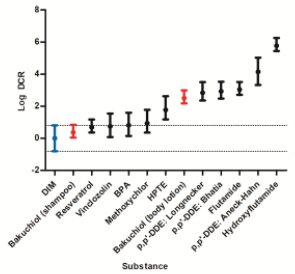
40

Dietary Comparator Ratios



Range indicates variability in exposure for both dietary comparator and test substances

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OXFORD JOURNALS Toxicological Sciences

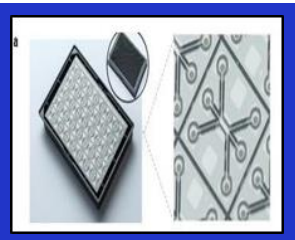
Toxicol Sci. 2019 Feb; 167(2): 375–384.
Published online 2018 Sep 22. doi: 10.1093/toxsci/kfy245
PMCID: PMC6358230
PMID: 30247711

Employing Dietary Comparators to Perform Risk Assessments for Anti-Androgens Without Using Animal Data

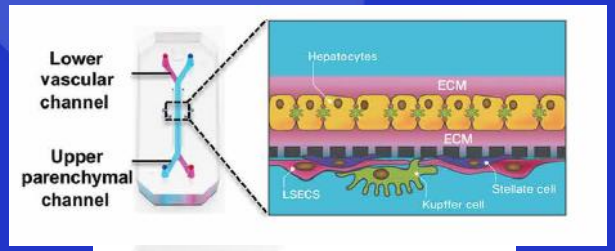
Microphysiological Systems (MPS)

TissUse

Mimetas

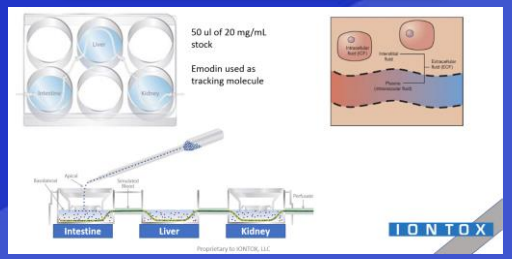


Emulate



CNBio

IonTox



Conclusions

Changing global environment for toxicology

- Consumers are demanding change; calls for non-animal, next generation risk assessments
- NGRA is a framework of non-standard, bespoke data-generation, driven by the risk assessment questions
- Enabling a transition from using data from tests in live animals to one founded on understanding the effects of chemicals in humans using computational approaches and *in vitro* methods that evaluate changes in biologic processes using human cells
- Constructed from *in silico* modelling approaches and *in vitro* solutions
- Need to ensure quality/robustness of the non-standard (non-TG) work and to characterise uncertainty to allow informed decision-making (BENCHMARKING)
- Shortcomings will be addressed by current and future research
- More research, creativity and examples needed to land this successfully with regulators

The NEW Gold Standard

Was:

- Rodents
- Pathology
- High-dose apical endpoints
- No adverse effect level
- Uncertainty factors

Is Now:

- Broad-based NAMs
- Implementing new NAMs
- Exposure led (PBK)
- **Bioactivity not pathology**
- **Protection not prediction**
- **Underpinned by Computational modelling**

Press Release 19 August 2021

EPA United States Environmental Protection Agency

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CONTACT US

EPA and Unilever Announce Major Research Collaboration to Advance Non-animal Approaches for Chemical Risk Assessment

August 19, 2021

Contact Information
EPA Press Office (press@epa.gov)

WASHINGTON – Today, the U.S. Environmental Protection Agency (EPA) and Unilever announced a collaborative agreement to better ways to assess chemical risks associated with consumer products. This agreement builds on prior cooperation between Unilever regarding New Approach Methods (NAMs), which are a promising alternative to conventional toxicity testing that are to reduce reliance on the use of animals.

EPA and Unilever have been jointly evaluating and using NAMs since 2015. This collaboration is helping EPA implement its New Methods Work Plan and is the foundation for new efforts to demonstrate that these novel approaches can help decision make protect consumers, workers and the environment.

"EPA is a pioneer in developing and applying NAMs to identify and quantify risks to human health, while reducing the use of an chemical toxicity testing," said **H. Christopher Frey, Deputy Assistant Administrator for Science Policy in EPA's Office of Re and Development**. "We are excited to continue the collaboration with Unilever, which enhances the robustness of our mutual to demonstrate the use of NAMs."

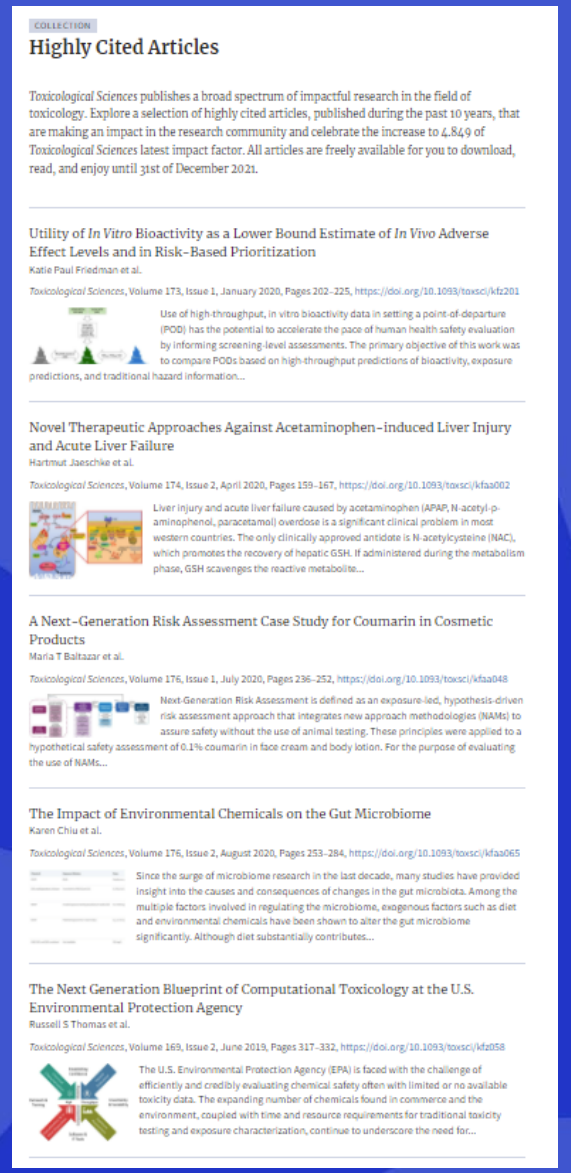
The new collaborative effort aims to establish a framework for the Next Generation of Risk Assessments based on NAMs. Such assessments are intended to quantify health risks to humans with sufficient scientific rigor to replace conventional animal-bas methods and to support EPA's mission to protect human health and the environment.

This collaboration will bring together more than $2 million in both monetary and in-kind contributions, including scientific expertise and equipment, to develop a comprehensive NAMs dataset for a minimum of 40 chemicals. The chemicals will be selected and grouped such

Thank you!

Supporting papers:
Toxicological Sciences 'Highly Cited Collection'
Click:
Highly Cited Articles | Toxicological Sciences | Oxford Academic (oup.com)

https://youtu.be/5Z2S8MnKp7g



COLLECTION

Highly Cited Articles

Toxicological Sciences publishes a broad spectrum of impactful research in the field of toxicology. Explore a selection of highly cited articles, published during the past 10 years, that are making an impact in the research community and celebrate the increase to 4.849 of *Toxicological Sciences* latest impact factor. All articles are freely available for you to download, read, and enjoy until 31st of December 2021.

Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization

Katie Paul Friedman et al.

Toxicological Sciences, Volume 173, Issue 1, January 2020, Pages 202–225, https://doi.org/10.1093/toxsci/kfz201

Use of high-throughput, in vitro bioactivity data in setting a point-of-departure (POD) has the potential to accelerate the pace of human health safety evaluation by informing screening-level assessments. The primary objective of this work was to compare PODs based on high-throughput predictions of bioactivity, exposure predictions, and traditional hazard information...

Novel Therapeutic Approaches Against Acetaminophen-induced Liver Injury and Acute Liver Failure

Hartmut Jaeschke et al.

Toxicological Sciences, Volume 174, Issue 2, April 2020, Pages 159–167, https://doi.org/10.1093/toxsci/kfaa002

Liver injury and acute liver failure caused by acetaminophen (APAP, N-acetyl-p-aminophenol, paracetamol) overdose is a significant clinical problem in most western countries. The only clinically approved antidote is N-acetylcysteine (NAC), which promotes the recovery of hepatic GSH. If administered during the metabolism phase, GSH scavenges the reactive metabolite...

A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products

Maria T Baltazar et al.

Toxicological Sciences, Volume 176, Issue 1, July 2020, Pages 236–252, https://doi.org/10.1093/toxsci/kfaa048

Next-Generation Risk Assessment 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. These principles were applied to a hypothetical safety assessment of 0.1% coumarin in face cream and body lotion. For the purpose of evaluating the use of NAMs...

The Impact of Environmental Chemicals on the Gut Microbiome

Karen Chiu et al.

Toxicological Sciences, Volume 176, Issue 2, August 2020, Pages 253–284, https://doi.org/10.1093/toxsci/kfaa065

Since the surge of microbiome research in the last decade, many studies have provided insight into the causes and consequences of changes in the gut microbiota. Among the multiple factors involved in regulating the microbiome, exogenous factors such as diet and environmental chemicals have been shown to alter the gut microbiome significantly. Although diet substantially contributes...

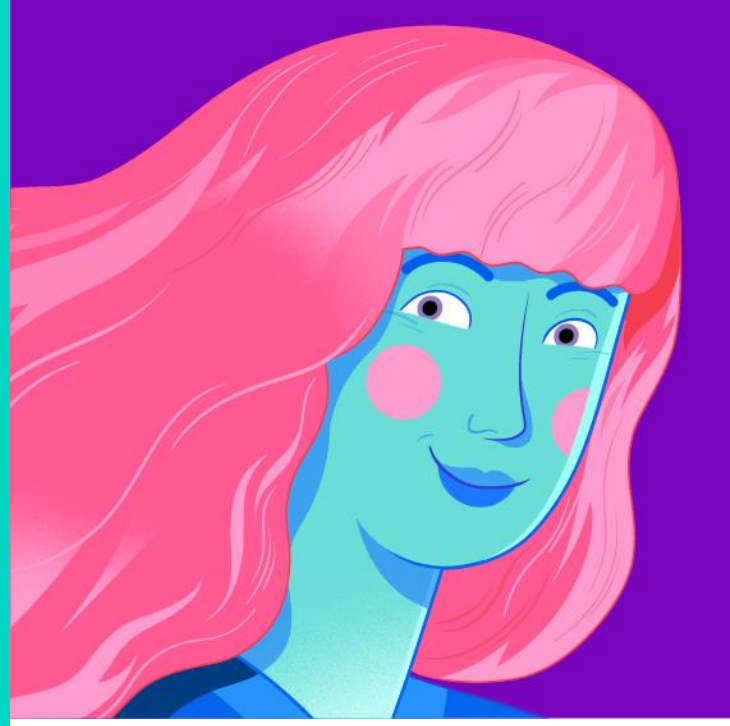
The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency

Russell S Thomas et al.

Toxicological Sciences, Volume 169, Issue 2, June 2019, Pages 317–332, https://doi.org/10.1093/toxsci/kfz058

The U.S. Environmental Protection Agency (EPA) is faced with the challenge of efficiently and credibly evaluating chemical safety often with limited or no available toxicity data. The expanding number of chemicals found in commerce and the environment, coupled with time and resource requirements for traditional toxicity testing and exposure characterization, continue to underscore the need for...

Decision making in Next Generation Risk Assessment (NGRA)



Using Computational Models to Make Sense of Complex Data

Unilever

07/11/2022

Learning objectives

- Understanding of how models are used to make predictions or analyse data in toxicology, and how they can be useful.
- Awareness of different modelling approaches currently used in risk assessment (e.g., Bayesian inference, physiologically based kinetic models etc), illustrated with examples taken from case studies.
- Understand how to get started using computational approaches to analyse data (including open access tools and other resources).

Unilever

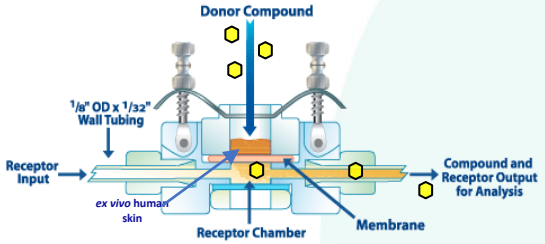
About me

- Degree in Mathematics from the University of Edinburgh
- PhD in Applied Mathematics from the University of Nottingham
- Postdocs in Germany at the University of Freiburg and the University of Heidelberg
- Joined Unilever in 2014, hired as a mathematical modeller
- Science leader in Computational Toxicology

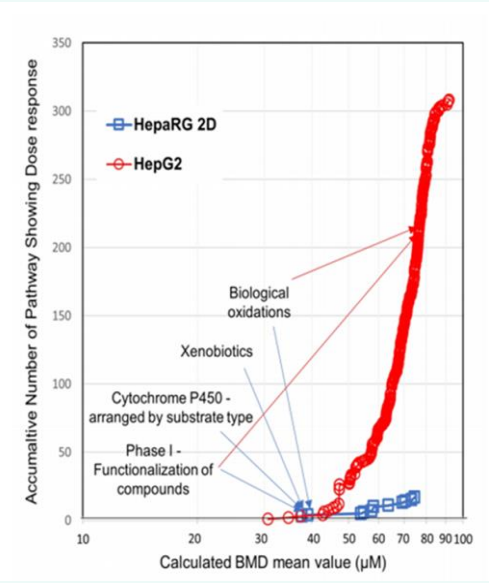
Unilever

Next Generation Risk Assessment is highly interdisciplinary

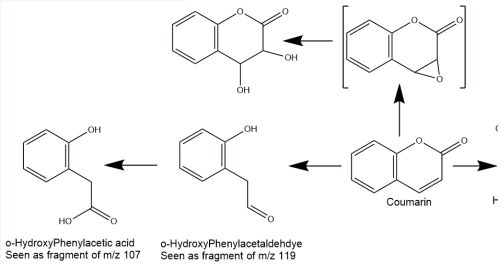
Risk assessment



Biology



Bioinformatics



Chemistry

$$y_t = \underbrace{\begin{bmatrix} w_{g,1}^{(1)} & \cdots & w_{g,1}^{(m)} \\ \vdots & & \vdots \\ w_{g,n_y}^{(1)} & \cdots & w_{g,n_y}^{(m)} \end{bmatrix}}_{C} \underbrace{\begin{bmatrix} \phi_g^{(1)}(x_t, u_t) \\ \vdots \\ \phi_g^{(m)}(x_t, u_t) \end{bmatrix}}_{\bar{\varphi}_g(x_t, u_t)} + e_t.$$

Mathematical and statistical modelling

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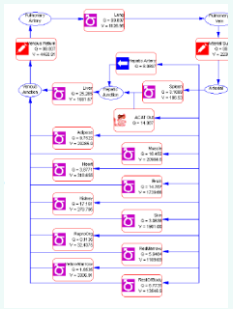
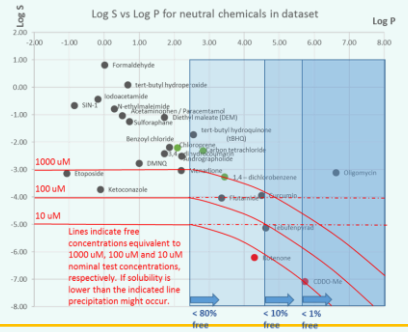
Back to the toolbox

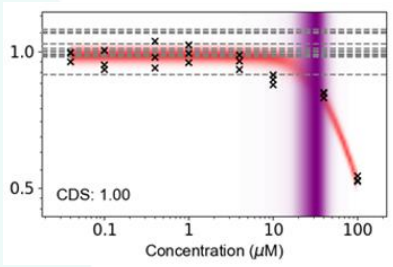
PBK models



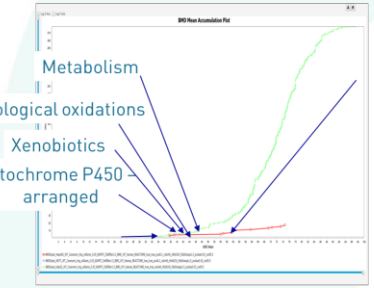
Free concentration



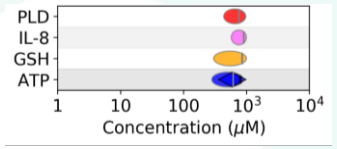
Conc. Resp. models



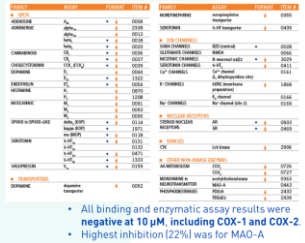
HTTr



CSP

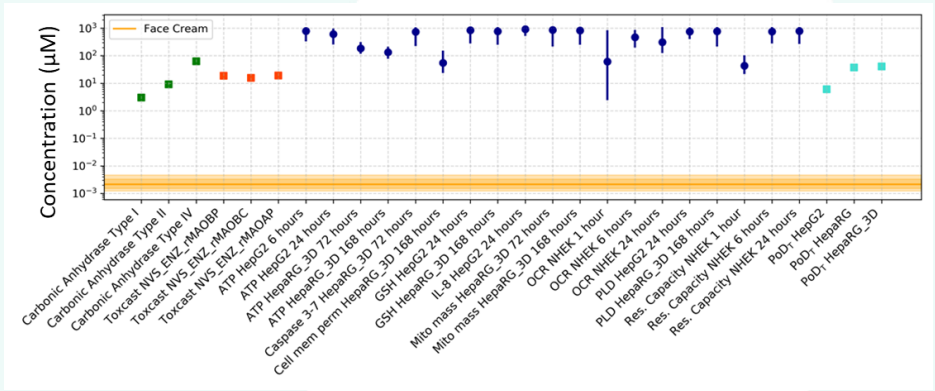


IPP



- All binding and enzymatic assay results were **negative at 10 μM, including COX-1 and COX-2.**
- Highest inhibition [22%] was for MAO-A

Bioactivity exposure ratio

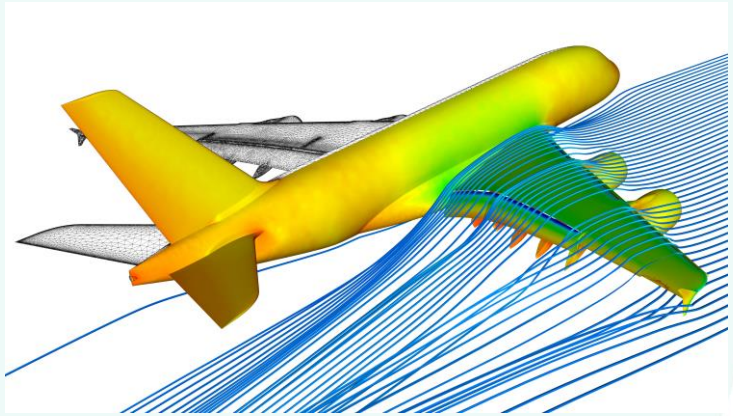


Inform safety decision

HTTr: High-throughput transcriptomics **CSP:** Cell Stress Panel **IPP:** In vitro pharmacological profiling

Computational models and their impact on everyday life

Air transport



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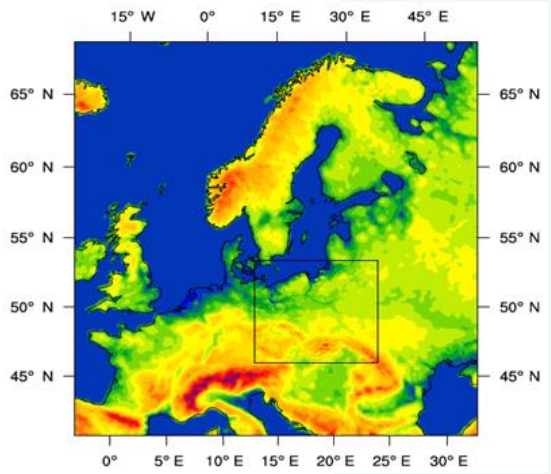
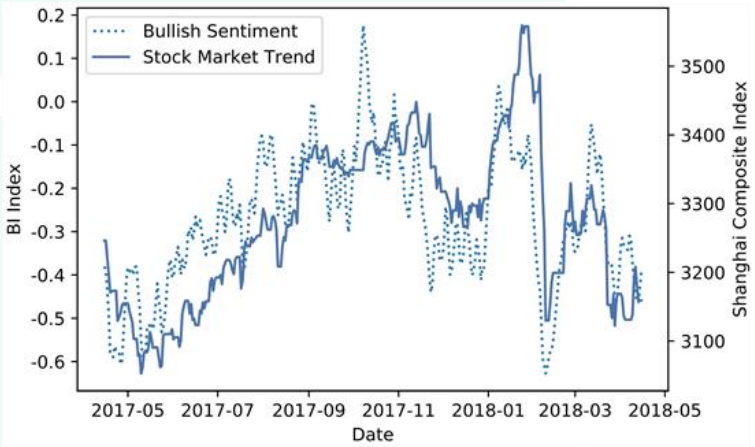
Weather forecast

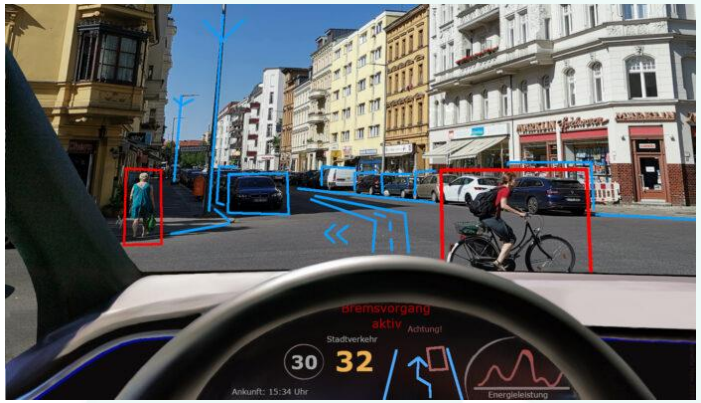


Satnav

Stock market



Self driving cars



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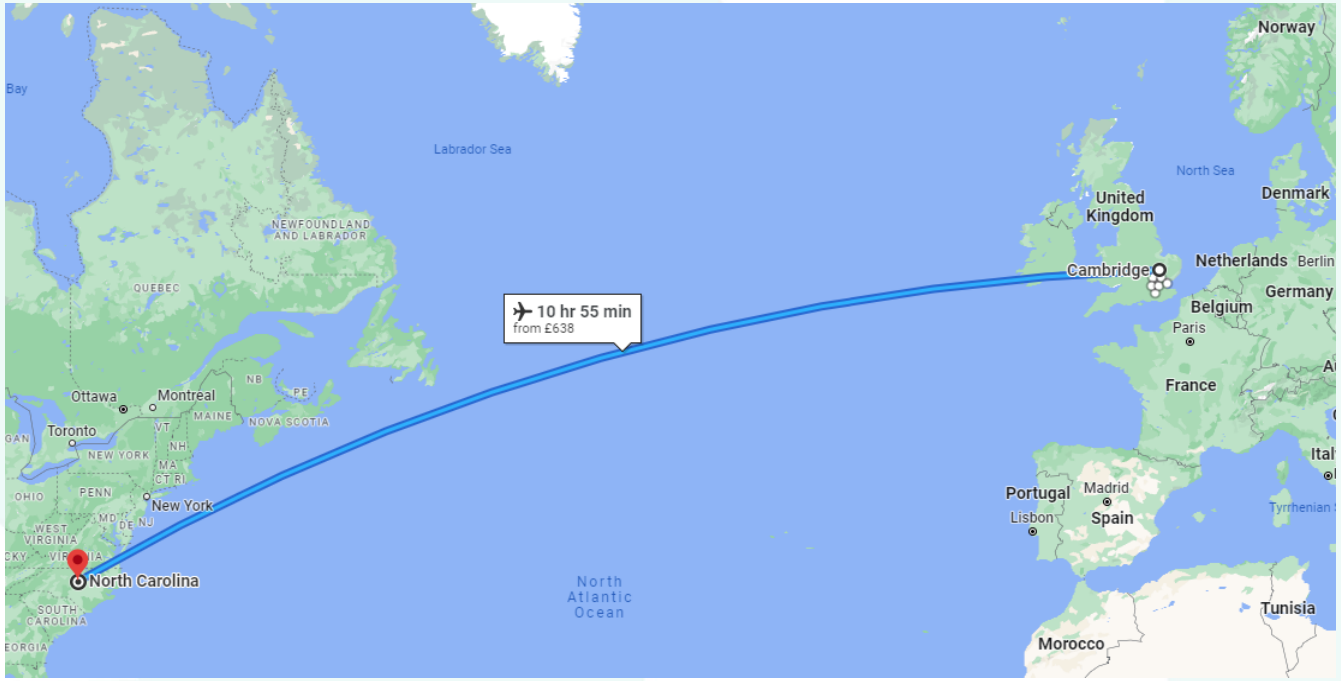
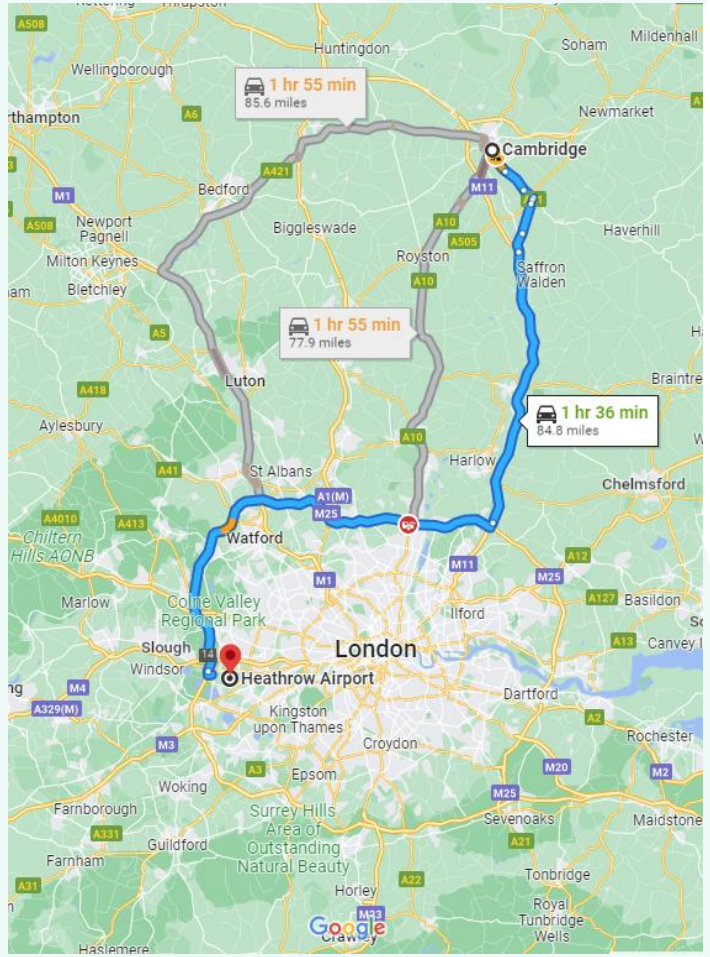
A simple example: my journey from the UK to the US





- How long will the journey take?
- How early should I leave?
- How much fuel will I need?

Unilever

Imagine a time before Google Maps…

What you want to know:

- Time it takes to get from home to the airport
- How early do you have to leave

What information you have:

- Distance from Cambridge to London
- Travel by car

Construct a (very) simple model:

- Model:

  Time = Distance/Speed
- ‘Data’:

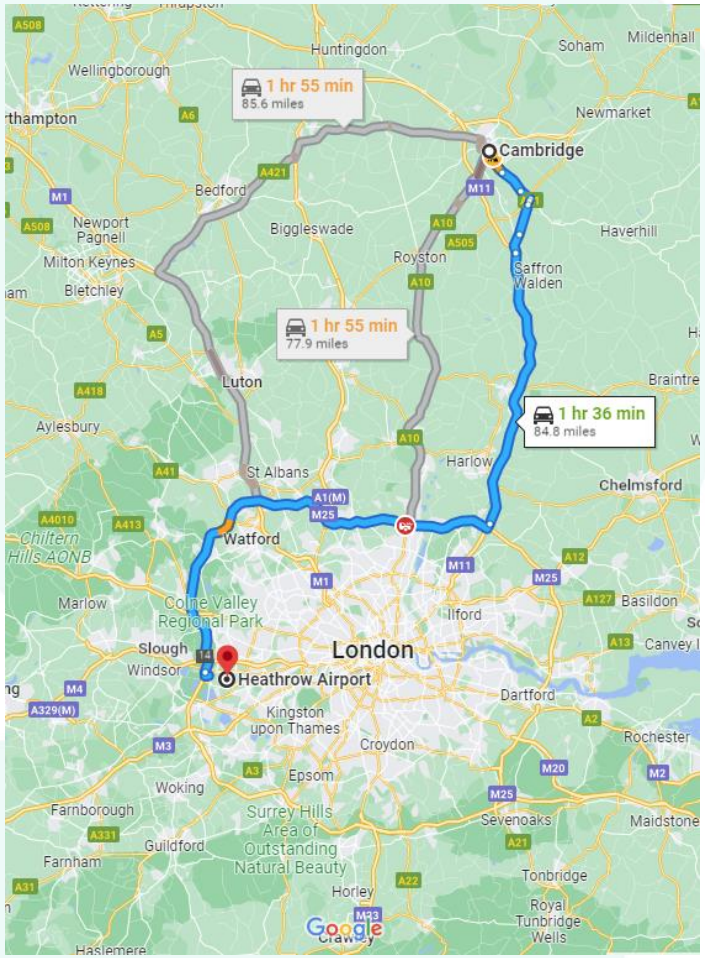
  Distance = 55 miles
- Assume:

  Speed = 60 miles per hour

Using the model make a decision

- You need to arrive to the airport by 12noon to catch your flight
- Based on your assumptions, your model prediction it will take 55 minutes
- Should you ‘trust’ the model and leave at 11.05?

Using models to make decisions

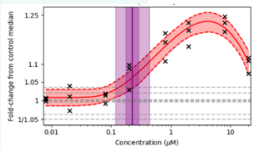


- Sitting behind Google maps is a far more complex and sophisticated set of models
- Informed by huge, complex datasets
- Provides estimation of journey time(s) based on route and time of day
- Even though it is more accurate, Google Maps can still go wrong!
- As a decision maker, both our model and Google Maps are potentially useful, but require judgement in terms of how you interpret their predictions.

Using these approaches together to make safety decisions

Hazard identification and characterisation of ingredients

Point of departure derived from concentration-response data

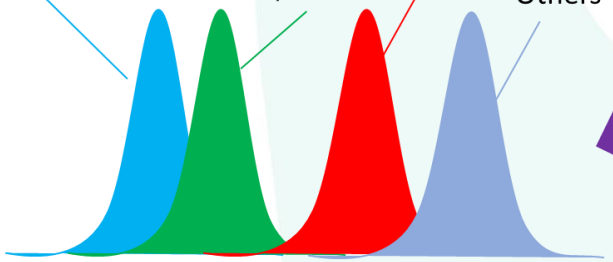


Cellular stress assays

Transcriptomics

Receptor binding

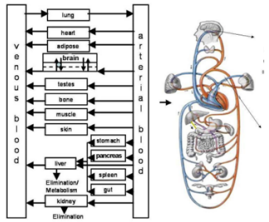
Others



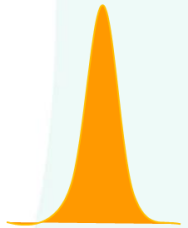
Consumer Exposure characterisation

Skin pen

Exposure models (PBK, free/total concentration)



Exposure estimation: Plasma C_{max}



Risk Assessment

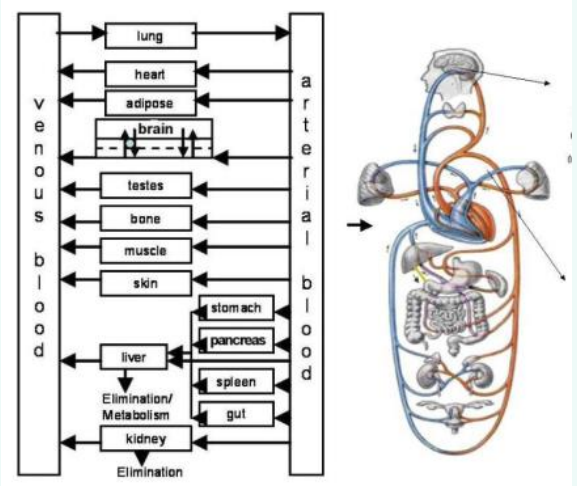
Calculation of Bioactivity Exposure Ratio (BER)

The BER/MoE is defined as the ratio of the PoD and the relevant exposure estimate

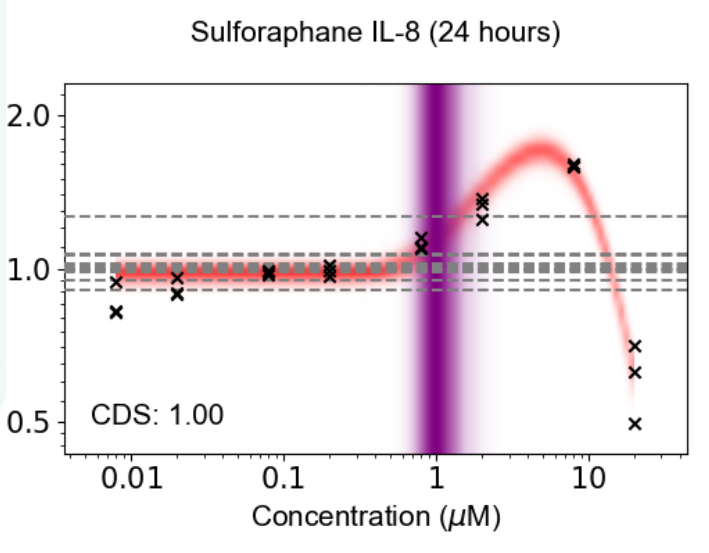
Unilever

Different types of computational approaches used in NGRA

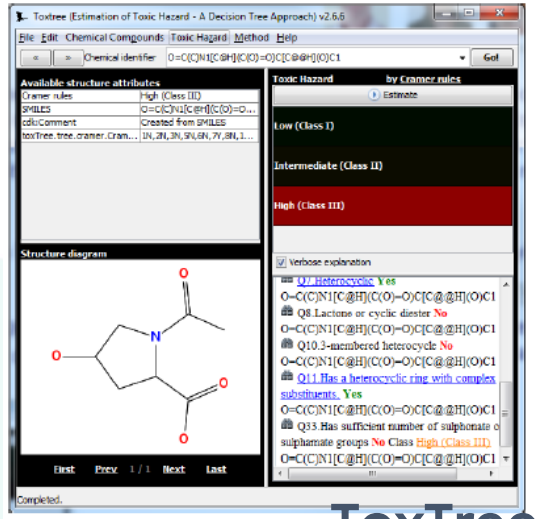
Physiologically-based kinetic (PBK) modelling



Dose response modelling

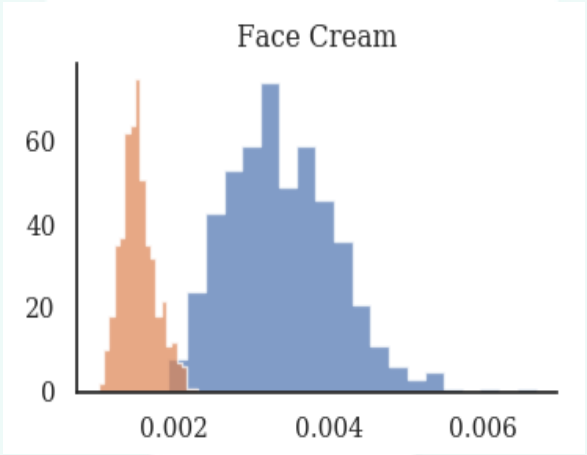


In silico tools

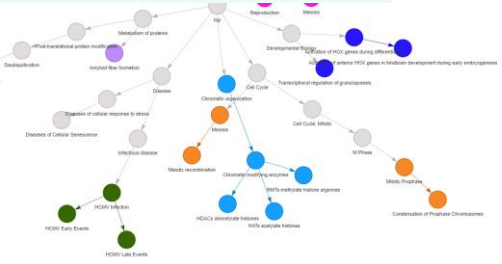


ToxTree

Statistical models of uncertainty and variability



Bioinformatics tools for analysing omics data



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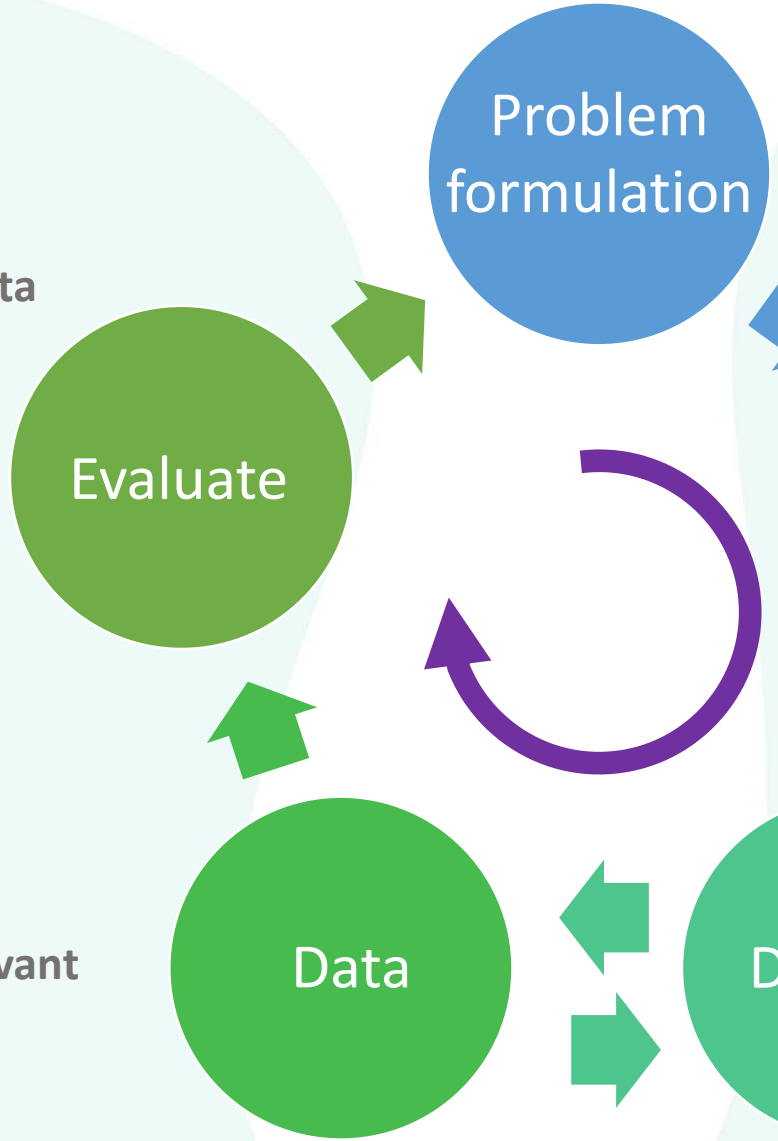
Principles of model development and the wet-dry cycle



- What question do you want to answer?
- What information do you have available?

Define model assumptions

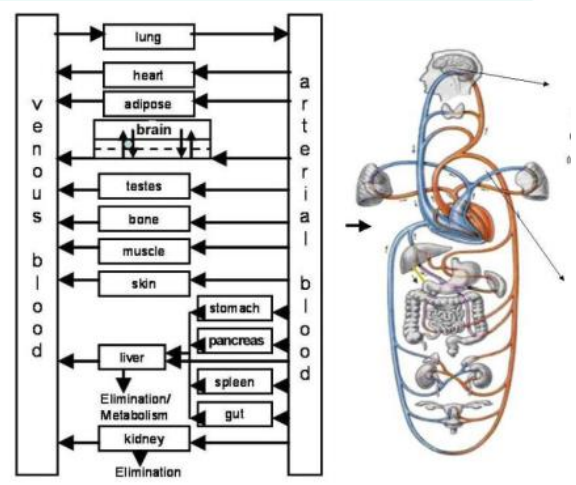
Develop and implement the model

Generate/curate relevant data

How does the model perform?
Does it describe the data well?

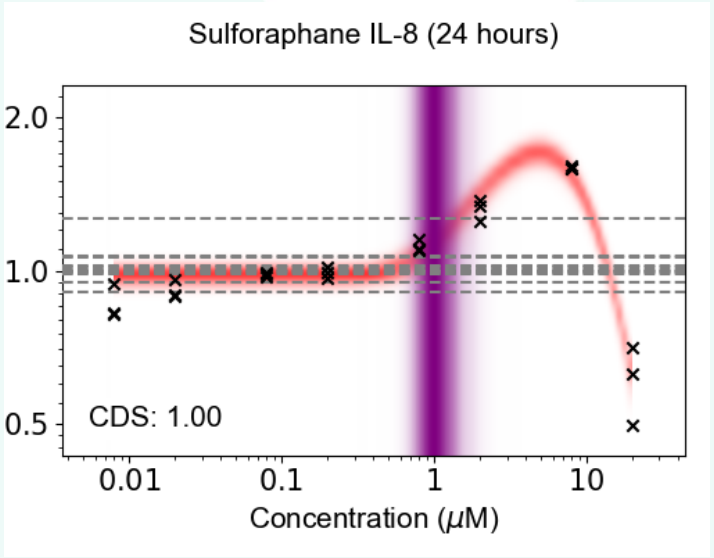
Two examples of computational models used NGRA

Physiologically-based kinetic (PBK) modelling



Example of **bottom-up** modelling approach

Dose response modelling



Example of **top-down** modelling approach

Physiologically based (Pharmaco*)kinetic models

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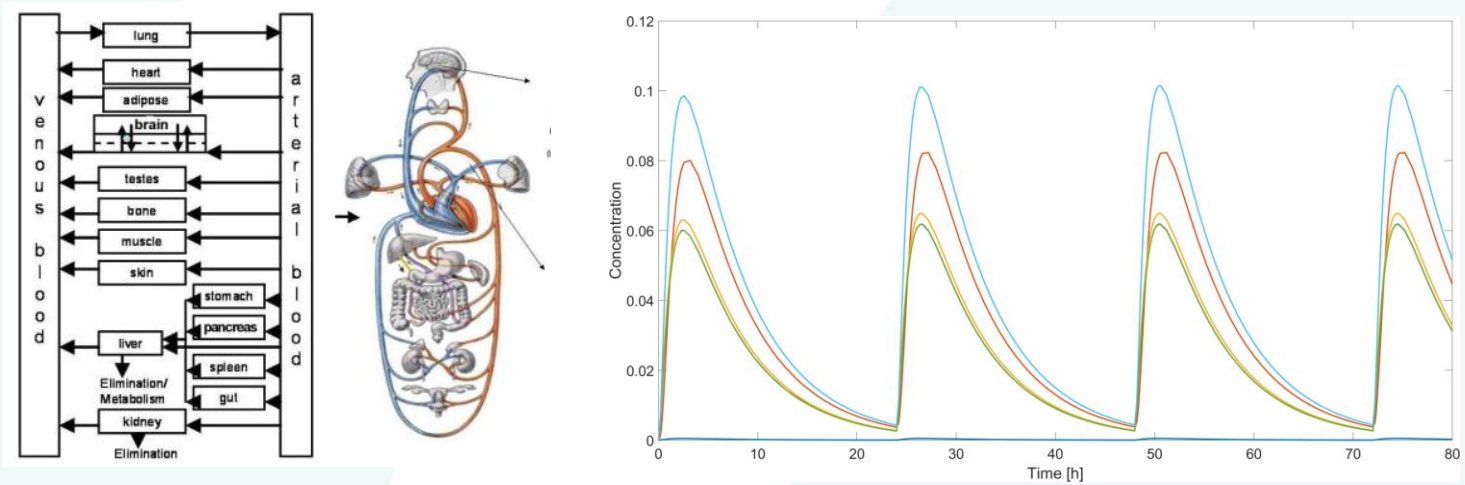
Physiologically-based (pharmaco) kinetic models



Problem: Quantify amount (e.g., concentration) of substance across different organs/regions of the body over **time** and for different **exposure routes**

Assumptions:

- Different regions of the body (e.g. organs) are divided into separate compartments
- Connection between compartments reflects physiology
- Movement of substances between compartments are governed by biophysical processes such as diffusion, perfusion, active transport etc

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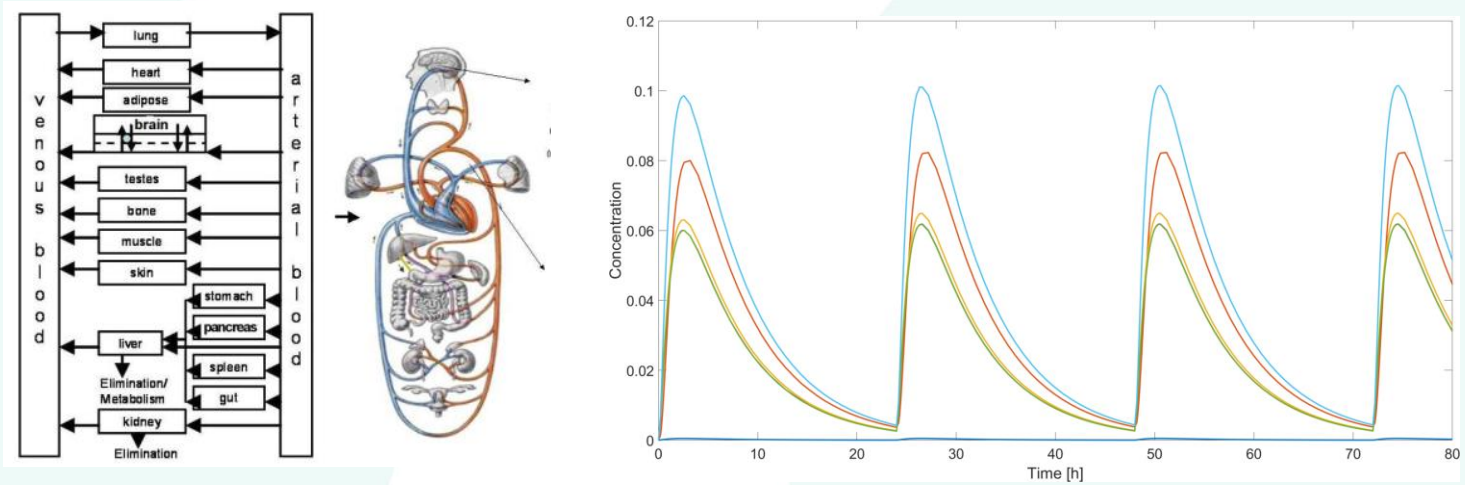
Physiologically-based (pharmaco)kinetic models



Develop

- Example equations:

Total blood flow rate into liver (mL/h)

Concentration in liver (ng/mL)

Metabolism

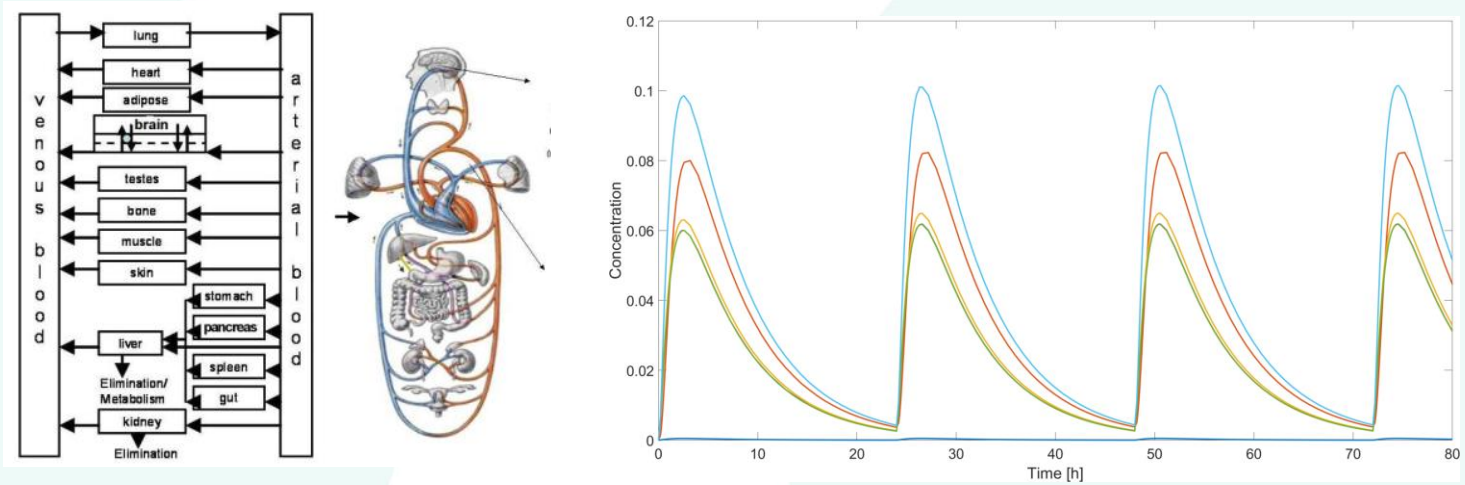
Rate of change of the amount (e.g. nanograms) of chemical in liver

$$V_{Liver}\frac{dC_{Liver}}{dt} = Q_{Liver}\left(C_V - \frac{C_{Liver}}{P_{Liver}}\right) - \frac{V_{max}C_{Liver}}{K_m + C_{Liver}}$$

Concentration in blood (ng/mL)

Liver:blood partition coefficient

Case study: Physiologically-based (pharmaco)kinetic models



Data:

- Information sources on model parameters:
  - *In silico* predictions
  - *In vitro* data (e.g. clearance rate)
  - Historical data (e.g. on physiological parameters such as weight/height distributions).
- Human PK data on measured concentration over time in plasma, urine etc

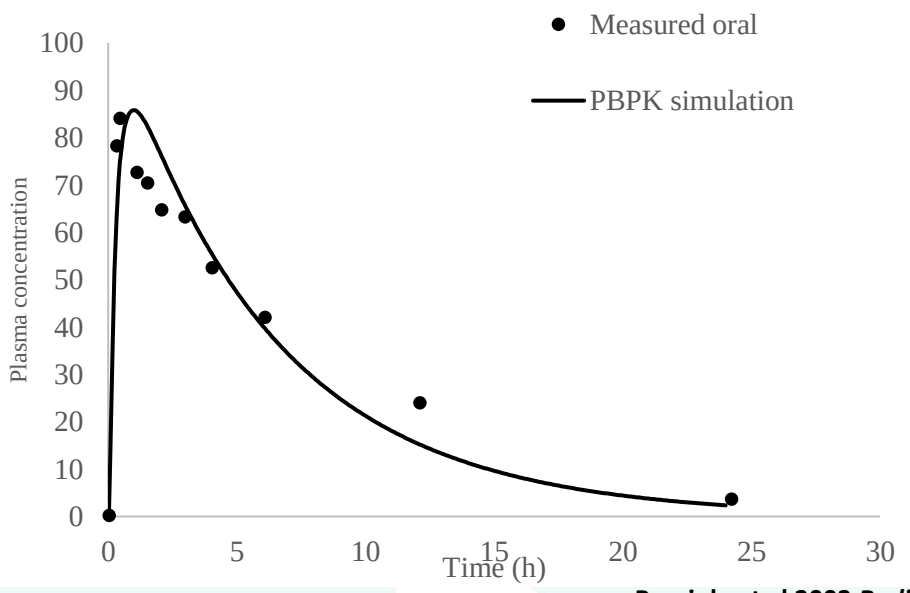
Unilever

Case study: Physiologically-based (pharmaco)kinetic models

Evaluate

- Compare model predictions against measured PK data
- Example:
  - Niacinamide used as face cream
  - Model parameters informed using *in silico* or *in vitro* data

Parameter	Value	Reference
LogP	-0.37	(Martin 1996)
pKa	13.39 (strongest acidic); 3.63 (strongest basic)	ChemAxon
Solubility	500000 mg/L (at 25 °C)	MERCK INDEX (1996)
Fraction unbound in plasma	0.82	Predicted (ADMET predictor)
human blood-to-plasma partition ratio	1.7	Predicted (ADMET predictor)
Vmax (CYP2E1)	60.14 pmol/mg min (In vitro human liver microsomes)	(Real, Hong, and Pissios 2013)
Km	2.98 mM	(Real, Hong, and Pissios 2013)
CL_{renal}	6.098 L/h	Predicted (GastroPlus) as glomerular filtration rate (GFR) x fraction unbound in protein (Fup)
Intestinal absorption: effective permeability (Peff cm/s)	5×10^4 cm/s	Fitted from oral human pharmacokinetic study (Bussink et al. 2002)

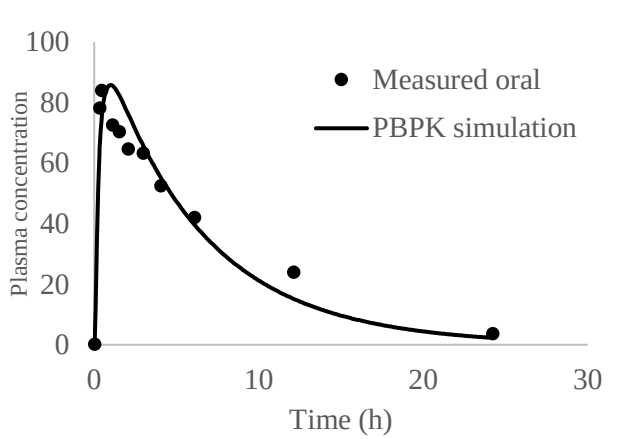


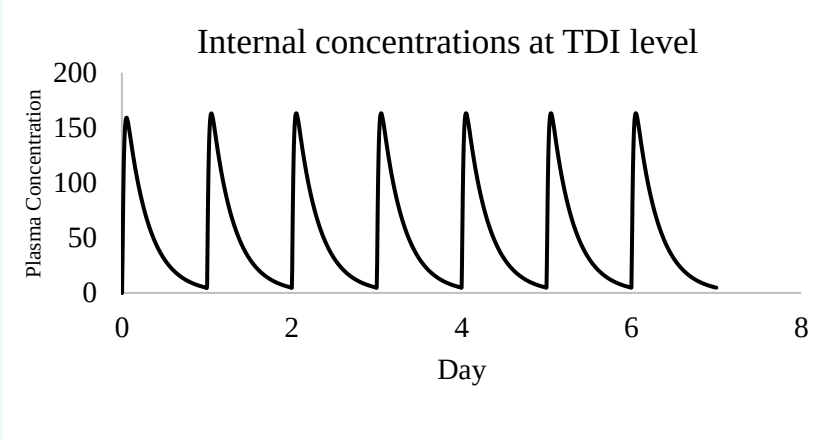
Bussink, et al 2002 *Radiotherapy and Oncology*

Hatherell, et al 2020 *Toxicological Sciences*

Case study: Physiologically-based (pharmaco)kinetic models

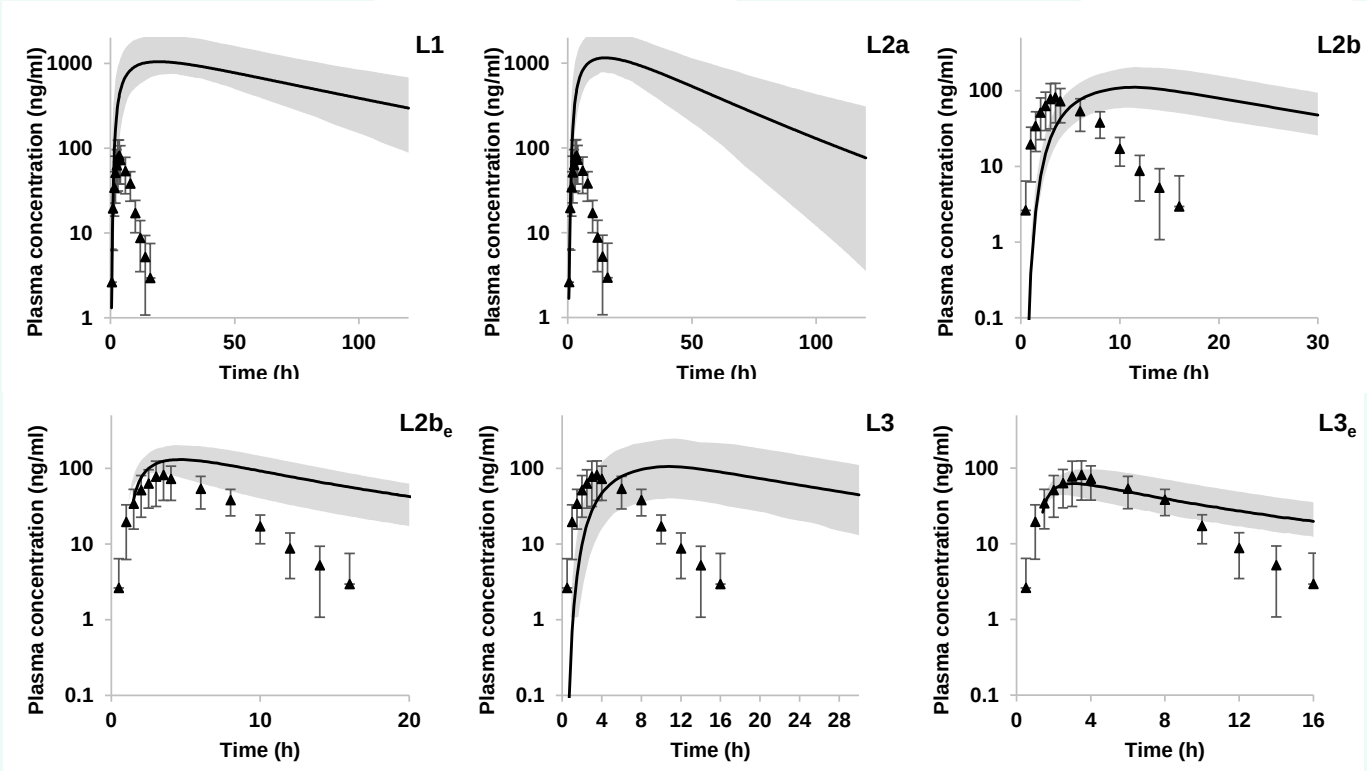
- Can use the model to then make predictions for other dosing regimes





Different parameterisation levels on model accurary

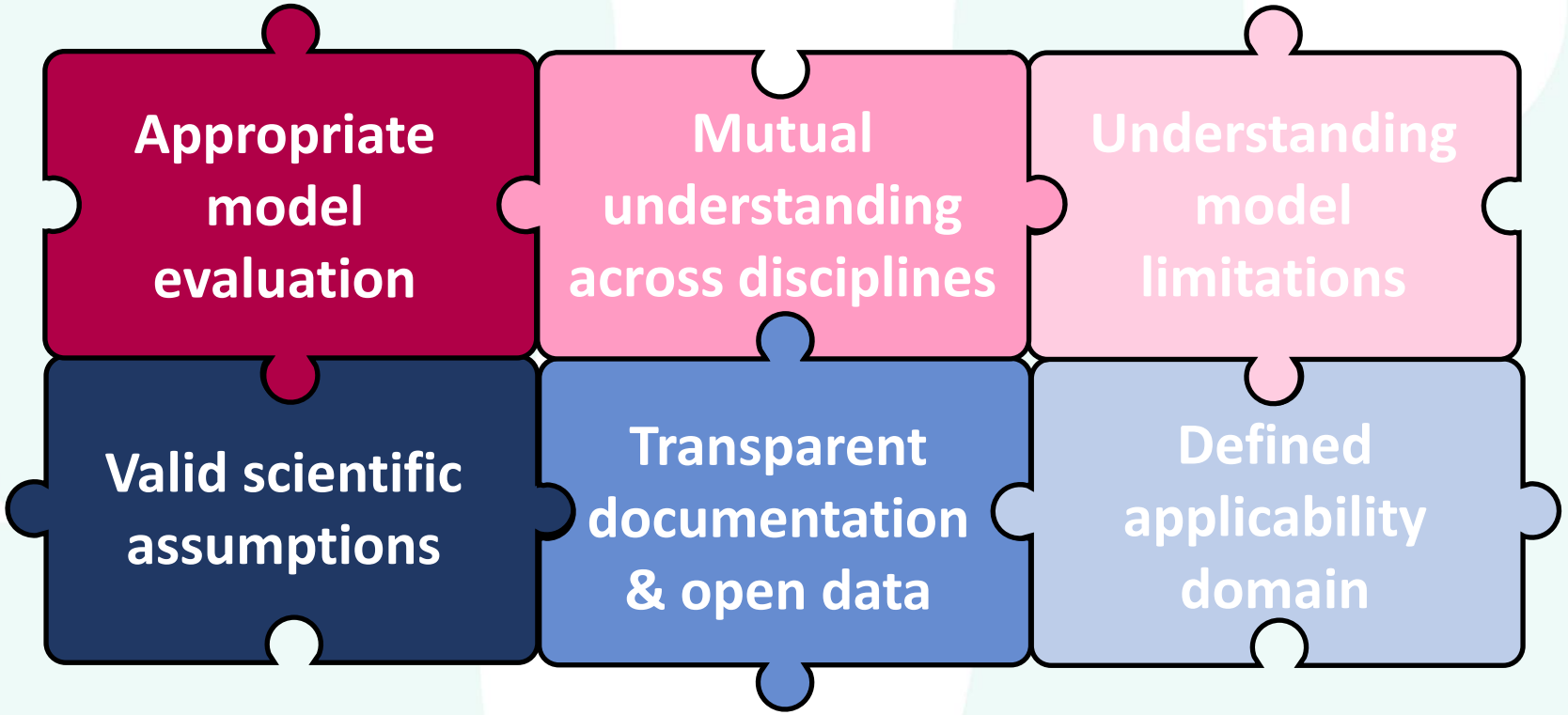
- Models will almost always be informed using imperfect data.
- Given the models are used for decision making, it is important to quantify **uncertainty** in how wrong the models can be



Li et al, (2022) PBK modelling of topical application and characterisation of the uncertainty of C_{max} estimate: A case study approach, Toxicology and Applied Pharmacology, Volume 442

Challenges in the acceptance of using computational approaches in NGRA

What do you think?



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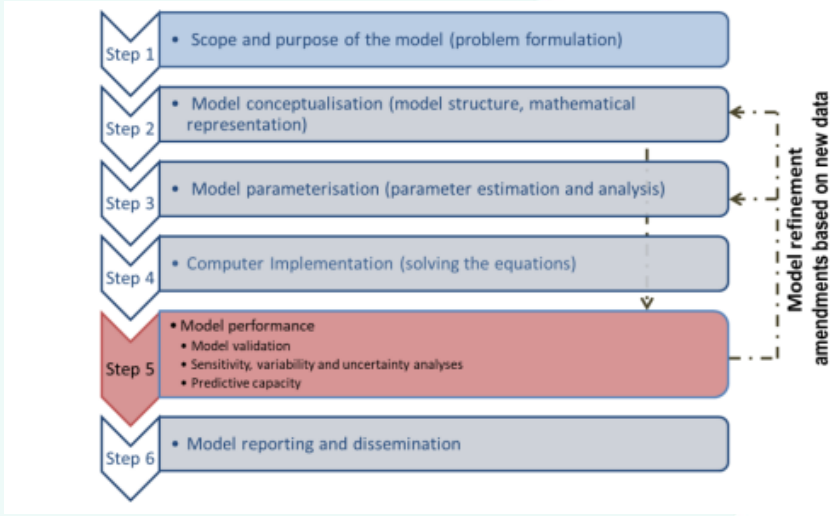
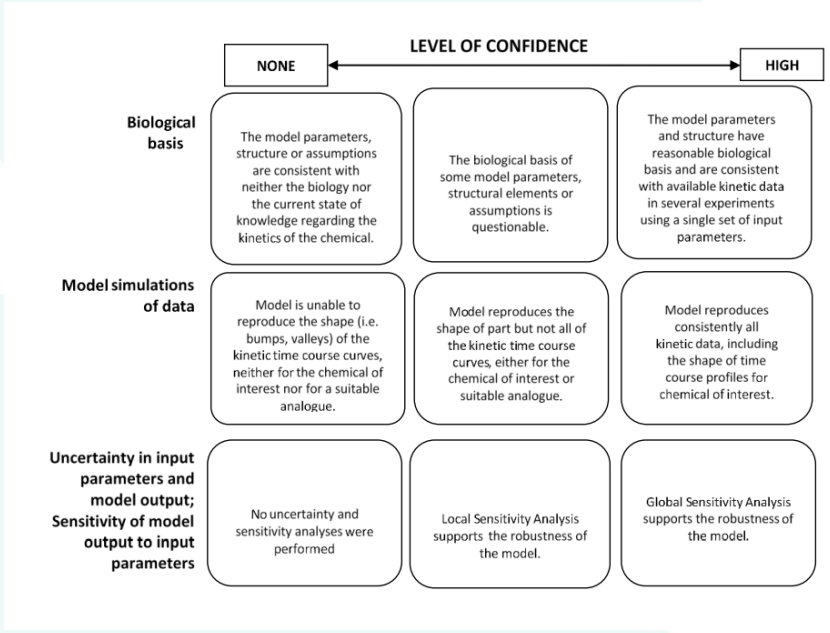
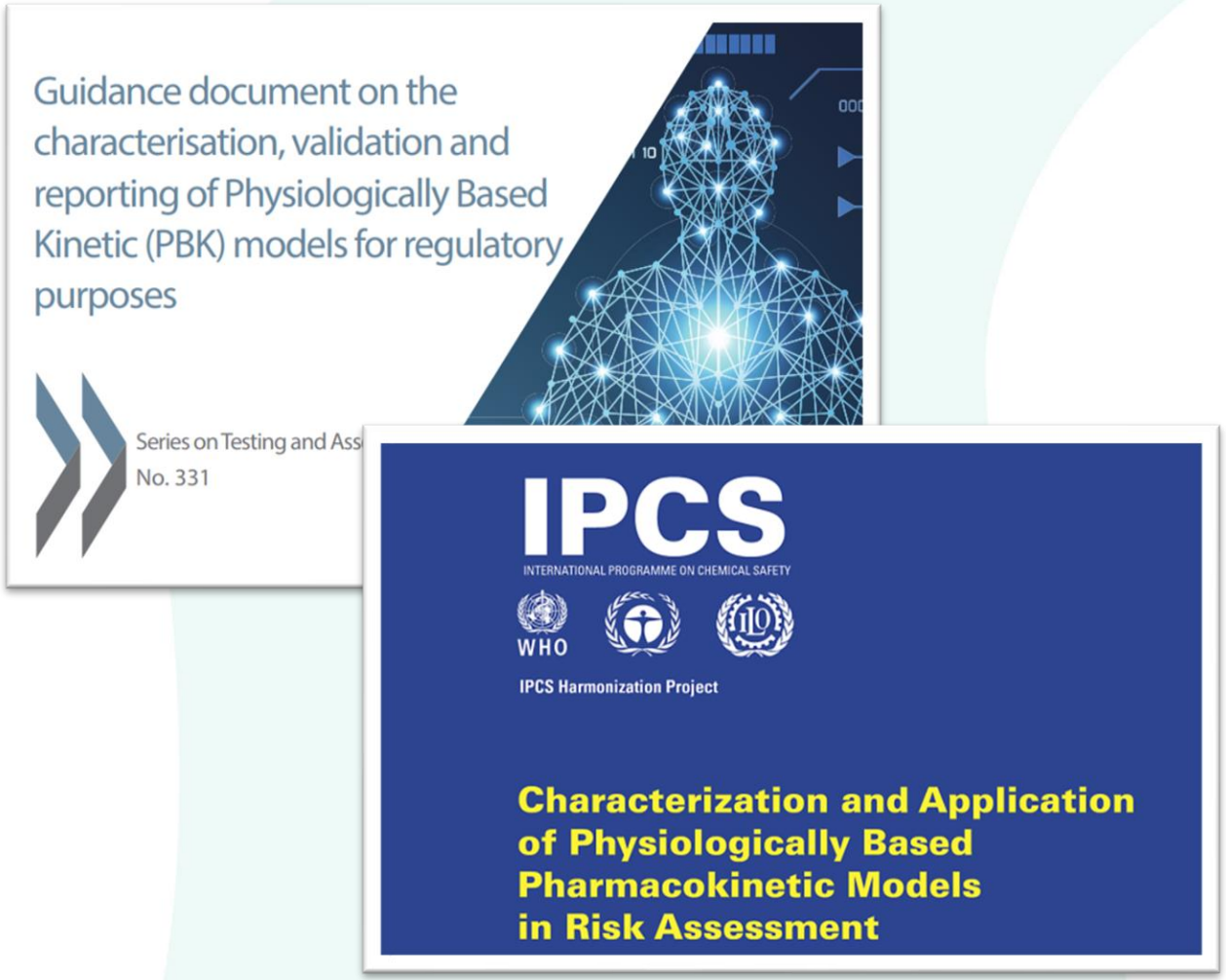
OECD guidance on best practice for PBK model development







- **https://www.who.int/publications/i/item/9789241500906**
- **https://www.oecd.org/chemicalsafety/risk-assessment/guidance-document-on-the-characterisation-validation-and-reporting-of-physiologically-based-kinetic-models-for-regulatory-purposes.pdf**

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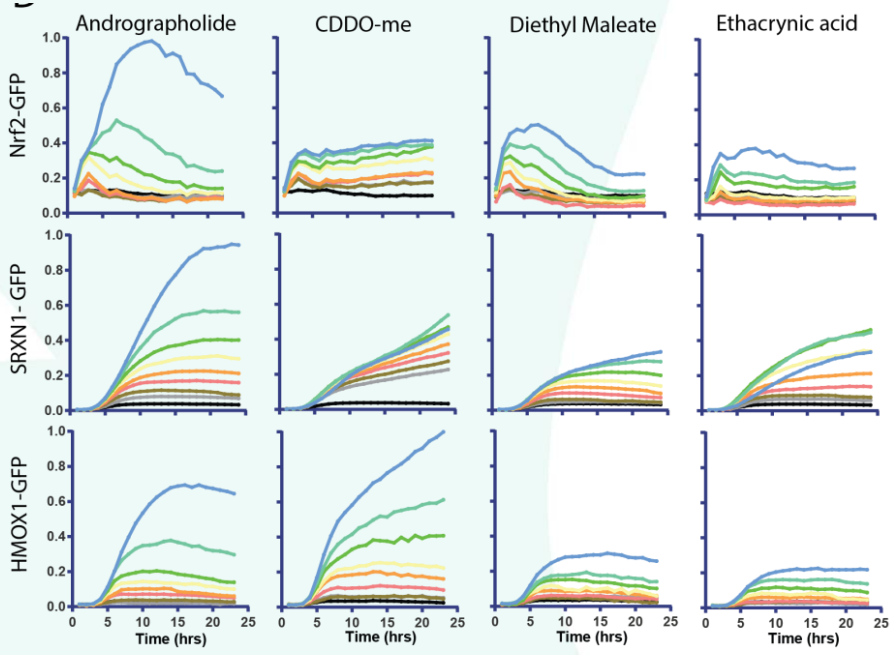
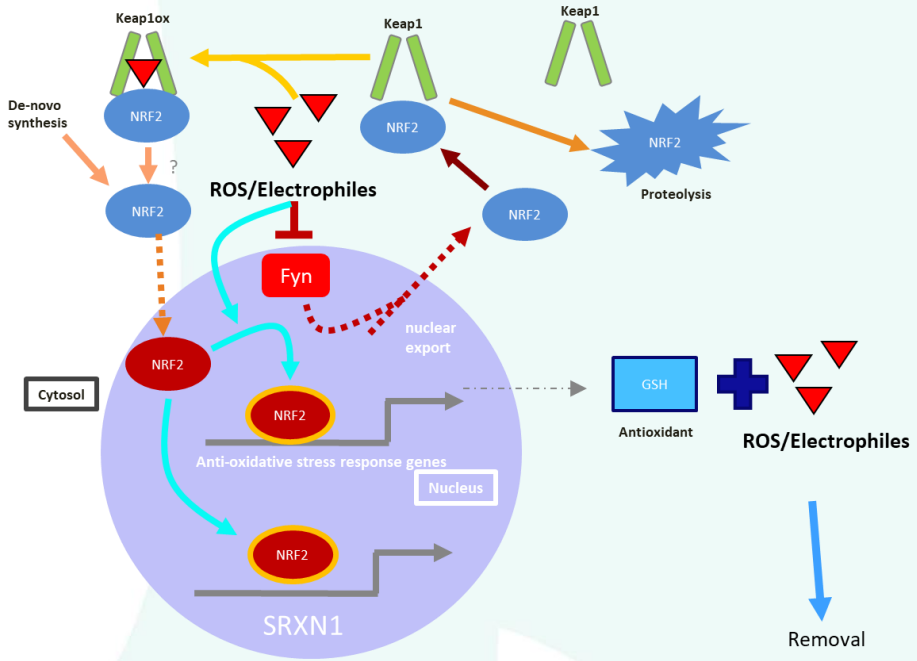
Going beyond PB(P)K models

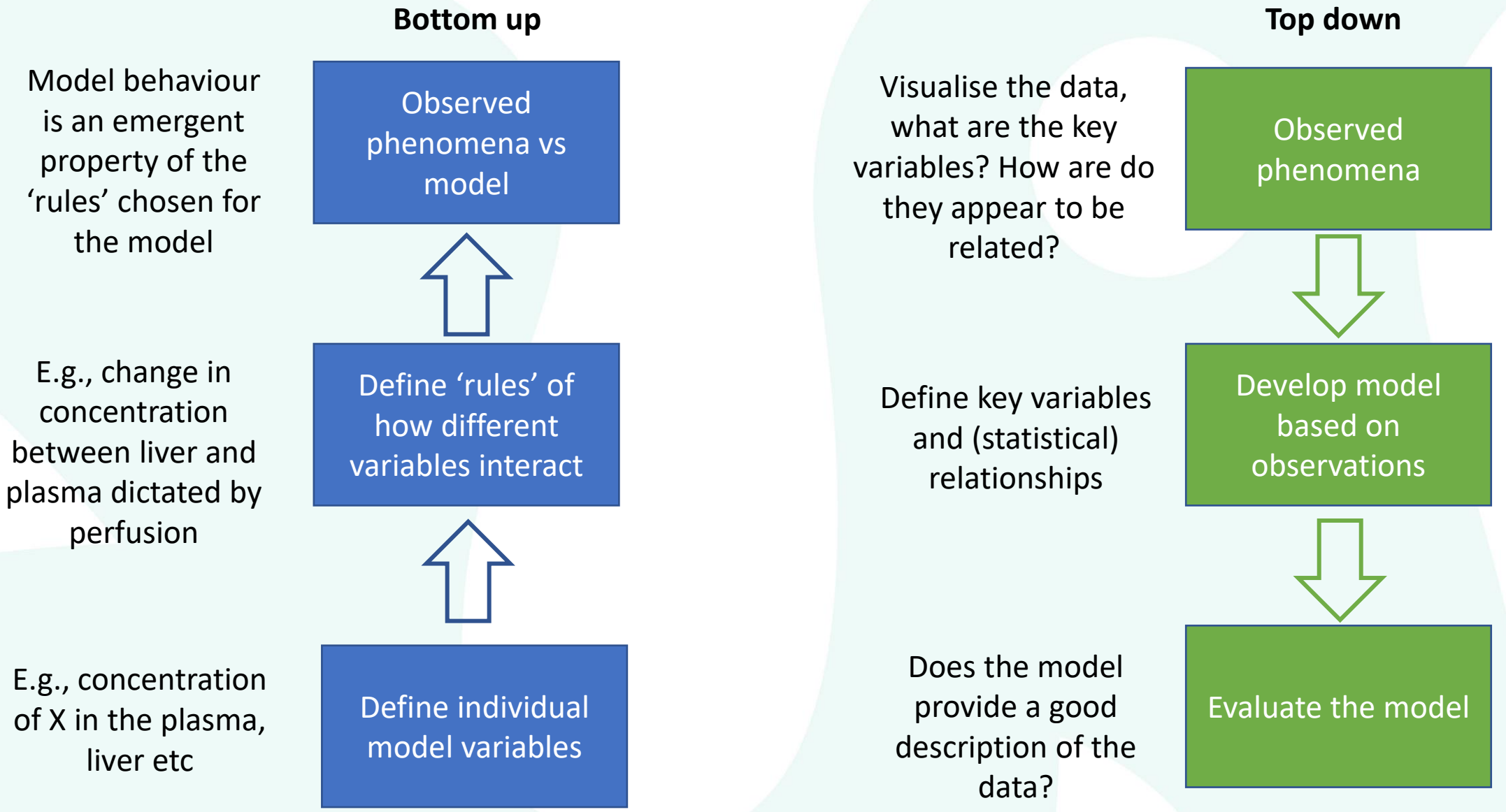
- The basic principles to bottom up modelling can be used in lots of other areas relevant to toxicology and risk assessment
- For example, for developing models of gene expression network or signalling pathways.
- The key challenge with these is there is limited data to decide on parameter or even equations.





Bas ter Braak et al, Mapping the dynamics of Nrf2 antioxidant and NFκB inflammatory responses by soft electrophilic chemicals in human liver cells defines the transition from adaptive to adverse responses (submitted)

Top down vs bottom modelling



Dose response models

The cell stress panel

Intended to cover off non-specific modes of action that lead to cell stress or mitochondrial toxicity

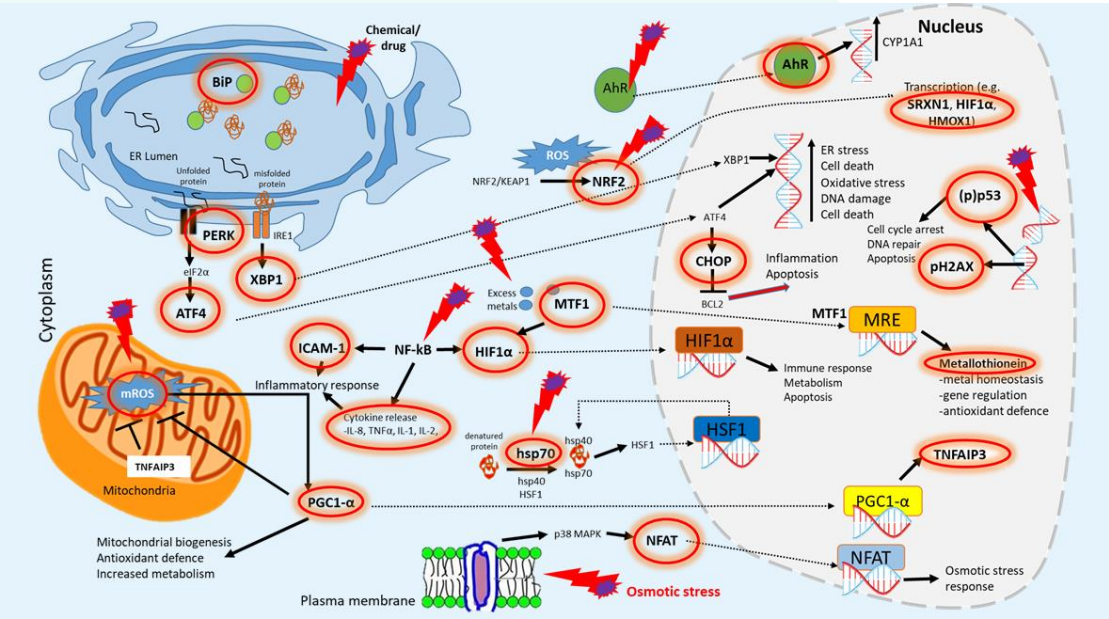


Image kindly provided by Paul Walker (Cyprotex)

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

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

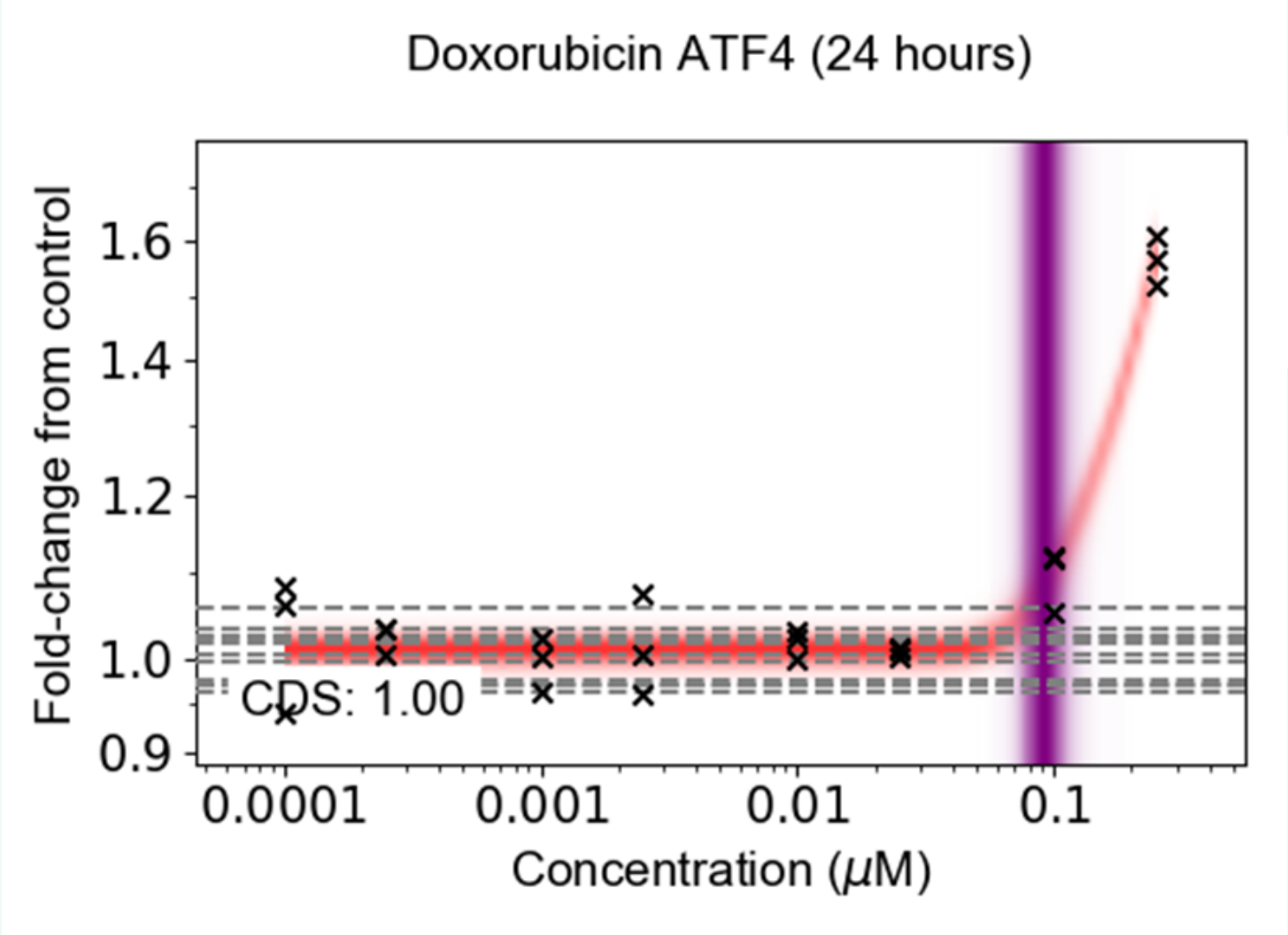
Sarah Hatherell,* Maria T. Baltazar,* Joe Reynolds,* Paul L. Carmichael,* Matthew Dent,* Hequn Li,* Stephanie Ryder,† Andrew White,* Paul Walker ,† and Alistair M. Middleton*,1

*Unilever Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, Bedfordshire

36 biomarkers identified that were representative of key stress pathways, mitochondrial toxicity and cell health.

Cell stress biomarkers predominantly measured using high content imaging. Includes Extracellular Flux assay to measure mitochondrial function.

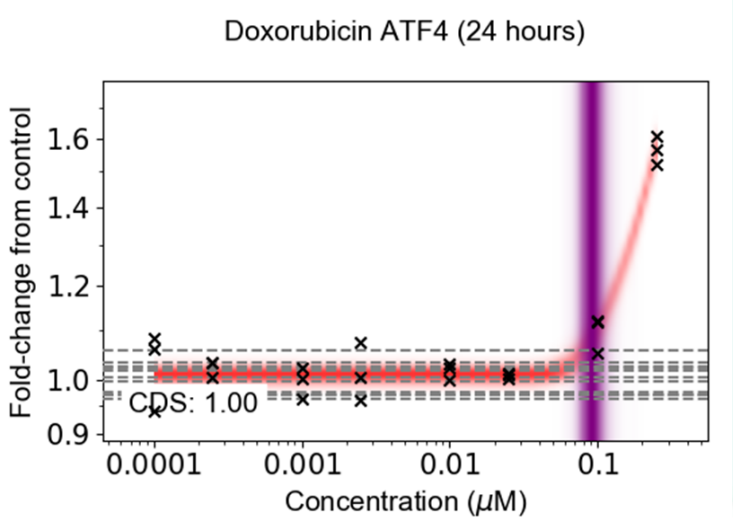
Dose response analysis and estimating PODs



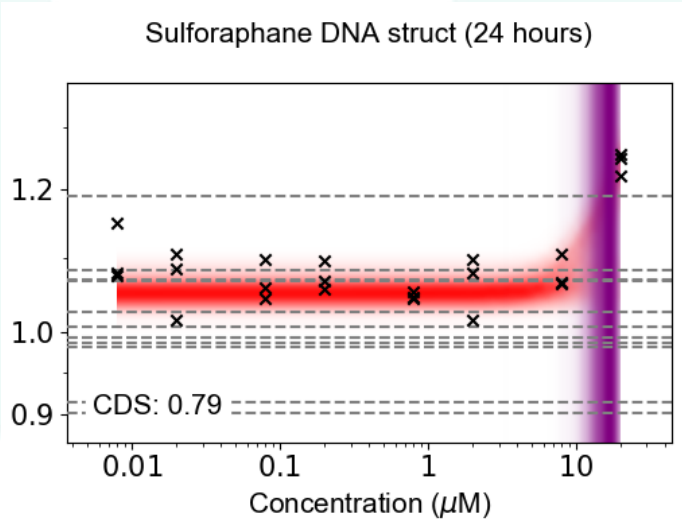
Hatherell *et al.*, 2020, Identifying and characterizing stress pathways of concern for consumer safety in next generation risk assessment, Tox. Sci. https://doi.org/10.1093/toxsci/kfaa054

Dose response analysis and estimating PODs

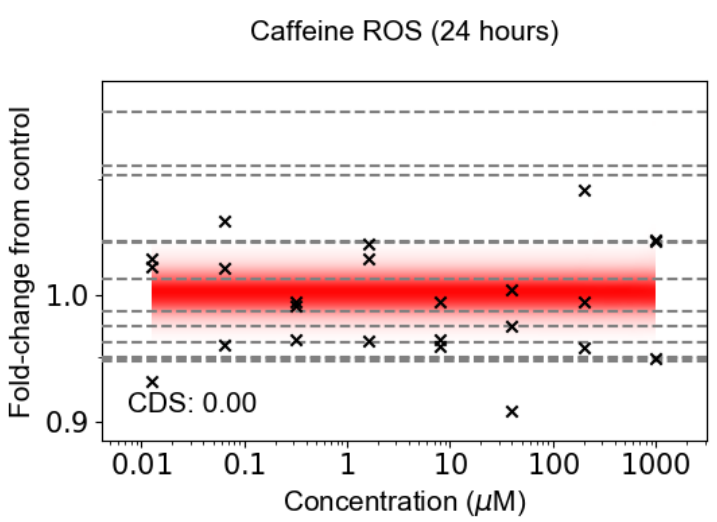
Clear effect



Is there an effect here?



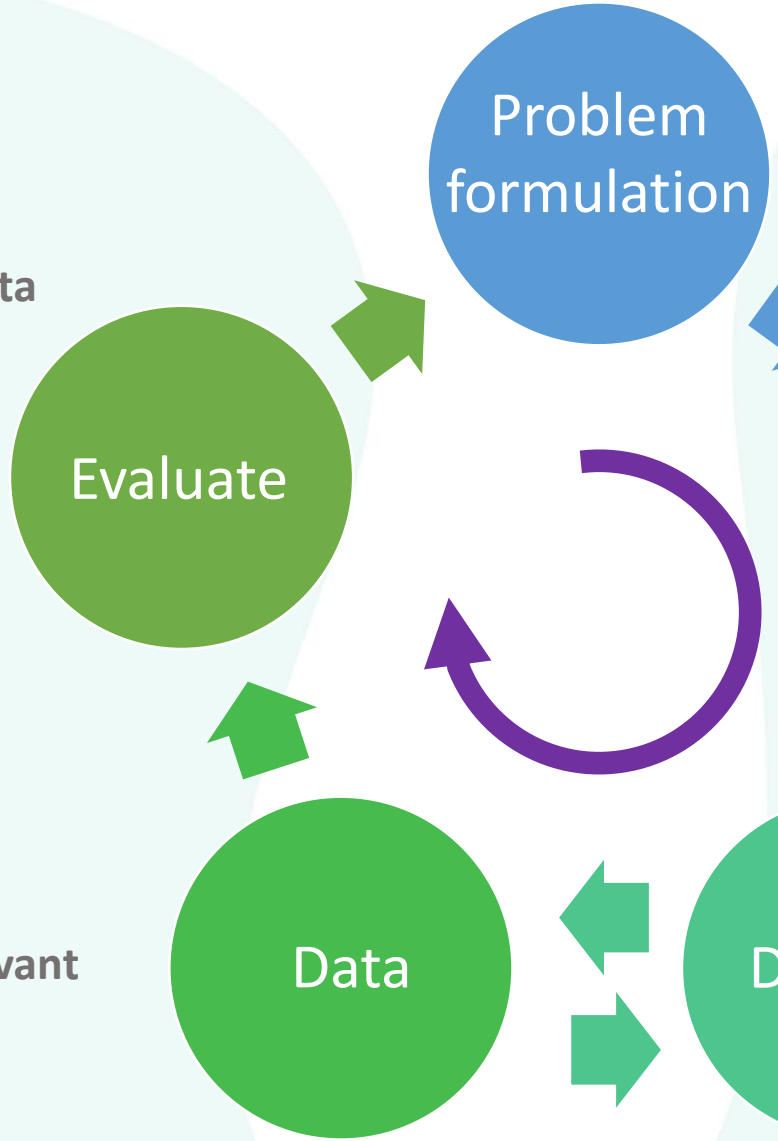
No effect



- Broadly, there are two approaches to doing this – **parametric** and **non-parametric**
- We will focus on the **parametric** approach

Hatherell *et al.*, 2020, Identifying and characterizing stress pathways of concern for consumer safety in next generation risk assessment, Tox. Sci. https://doi.org/10.1093/toxsci/kfaa054

Principles of model development and the wet-dry cycle



- What question do you want to answer?
- What information do you have available?

Define model assumptions

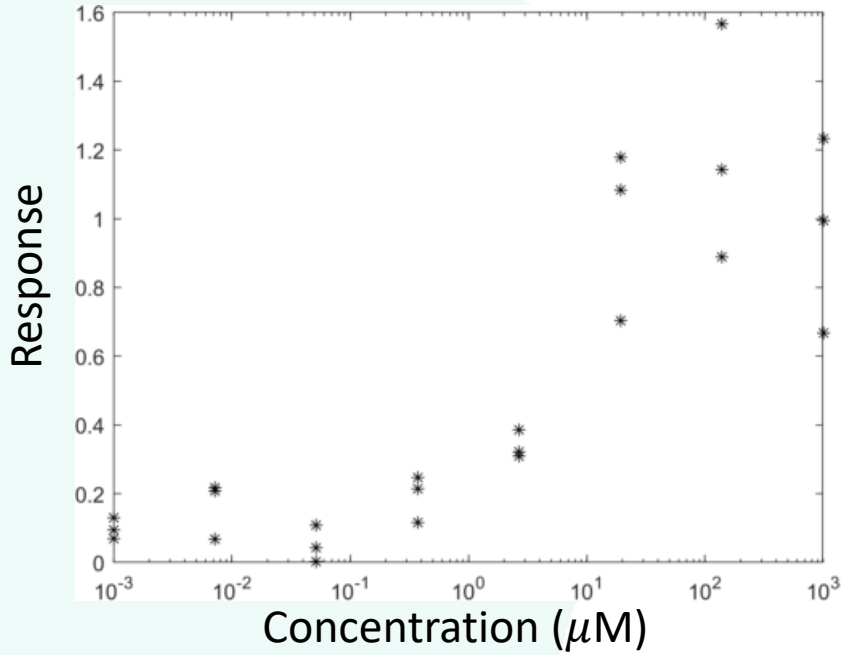
Develop and implement the model

Generate/curate relevant data

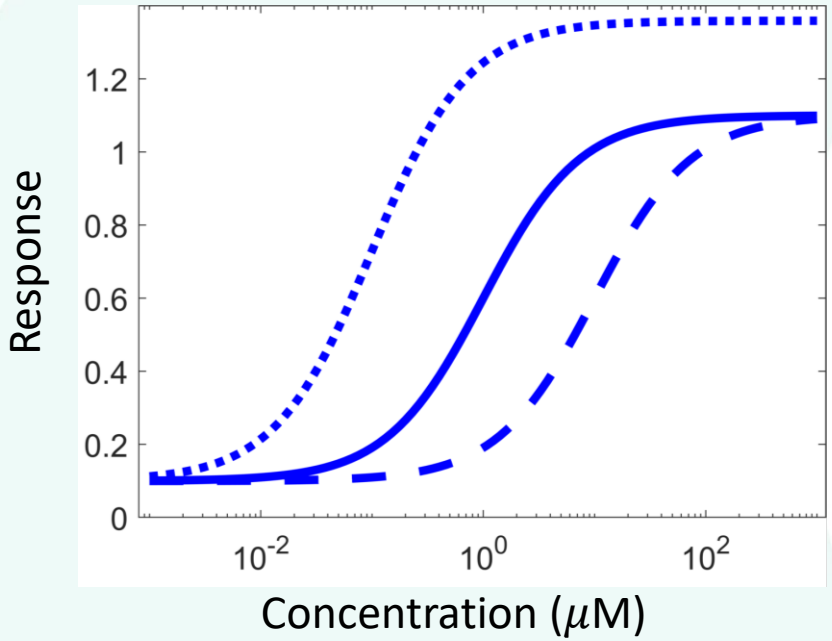
How does the model perform?
Does it describe the data well?

Developing a dose response model

Example dose response data

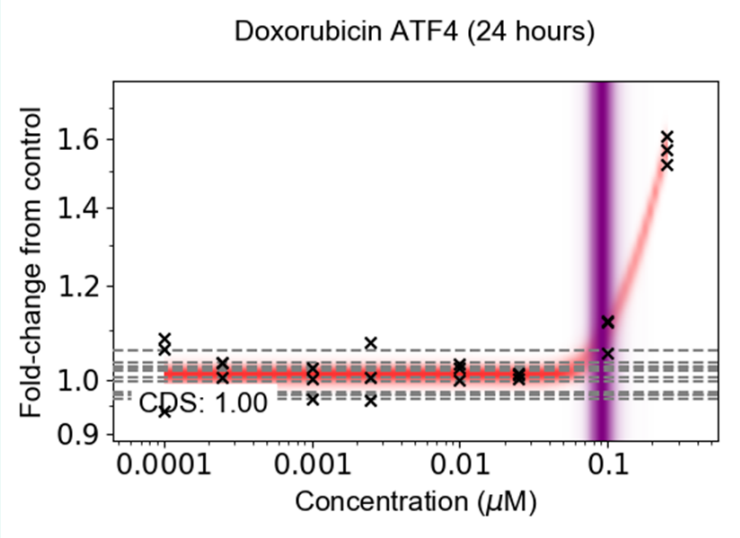


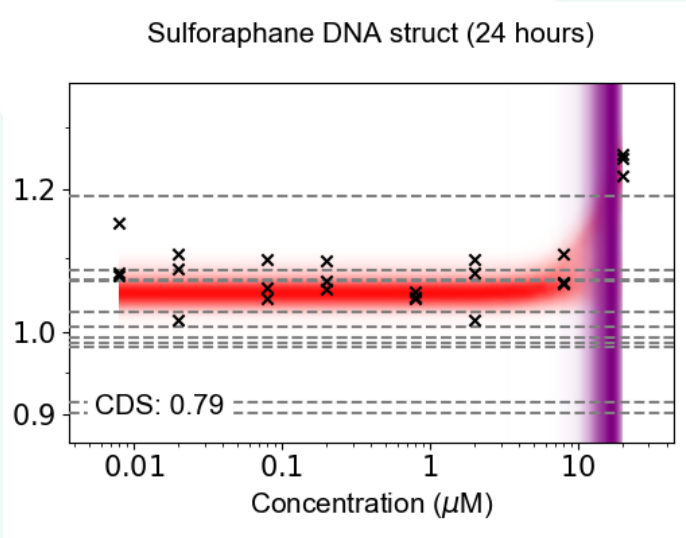
Hill function

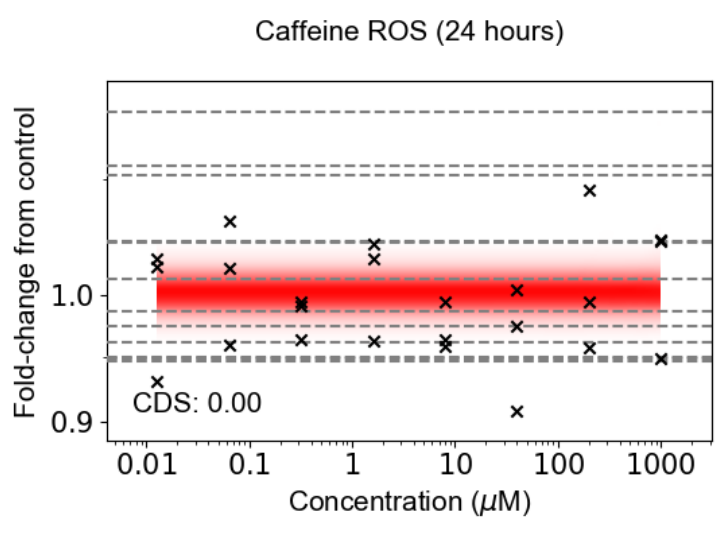


- **Problem**: We want to know:
  - Does the chemical have an effect on our biomarker
  - At what concentration does this occur?
  - We want to quantify the uncertainty in these.
- **Assumption**: There is an increase in our biomarker, which can be captured using a Hill function.

Bayesian statistics – what and why







- We want to quantify uncertainty in whether a certain event occurs, e.g.
  - Whether there is a concentration-dependent effect.
  - Whether you will reach the airport in 2 hours.
- One way to do this is through Bayesian statistics – our current approach to NGRA uses it a lot!
- Here, 'the probability' is a number that reflects the **plausibility** of some event occurring based on some data.

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Bayesian statistics – what and why

Bayesian probability:

- Probability reflects the **plausibility** or **belief** in some event being true.
- Provides framework for updating plausibility based on available data.
- For example, can talk about the **probability of a hypothesis being true**, or a parameter taking on a certain value.
- Key terms: credible interval, priors, posterior

Frequentist probability

- What people are normally taught in school
- Basis for **p-values** and **hypothesis testing**
- Probability reflects the relative frequency at which an event occurs in many over many repeated trials.
- Only really relevant when dealing with **well-defined random experiments**
- Can't use it to talk about the probability of a 'parameter taking a certain value' or a 'hypothesis being true'.

Thomas Bayes, 1701-1761

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Bayesian statistics – what and why

Bayesian interpretation of probability

- Probability quantifies the plausibility of some event.
- **Bayes' theorem**:

Likelihood

Prior

Posterior

$$P(X|D) = \frac{P(D|X)P(X)}{P(D)}$$

- Here, D is the data and X is random variable
- E.g., X – V_{max} parameter, D – experimental observations
- The key things are the likelihood, the prior and the posterior:
  - **Posterior**: probability that V_{max} takes a certain value
  - **Likelihood**: probability of the data, given V_{max}
  - **Prior**: probability reflecting initial assumptions V_{max}

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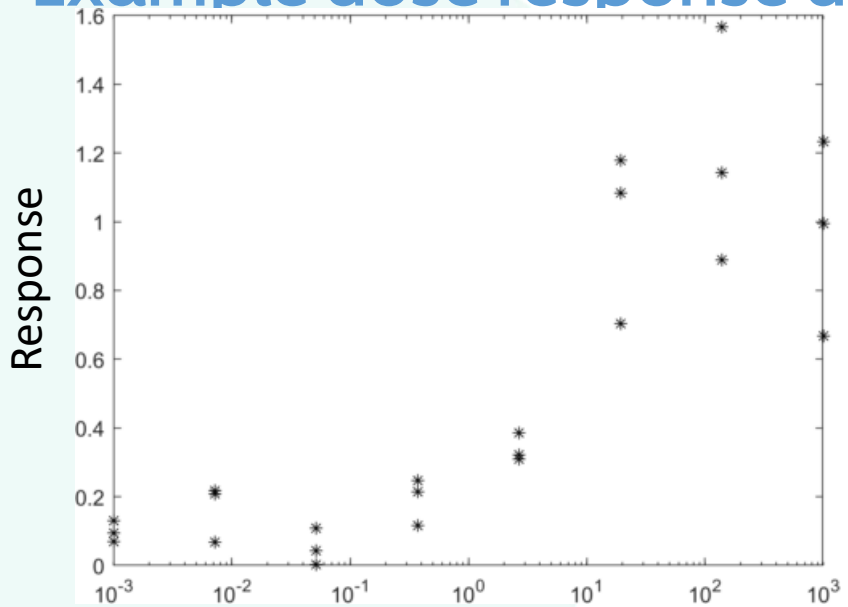
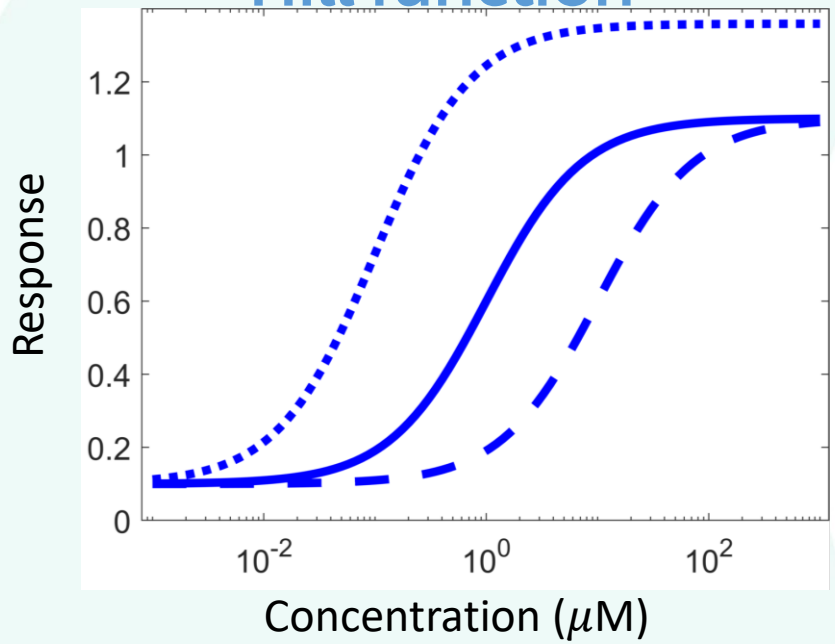
Back to the dose response example

Example dose response data



Hill function



Develop

- Main building blocks of the model:
  - Measured data = Mean Response + Observational Noise
  - $y = f(x|C, \theta, V_{max}) + \eta$
- y and x are the observations and concentrations respectively.
- Assume η is normally distributed with standard deviation σ

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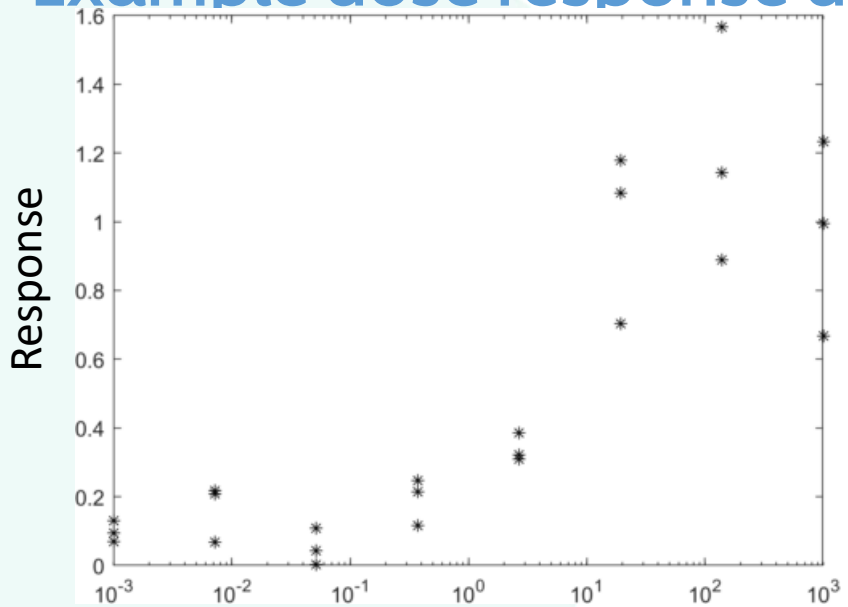
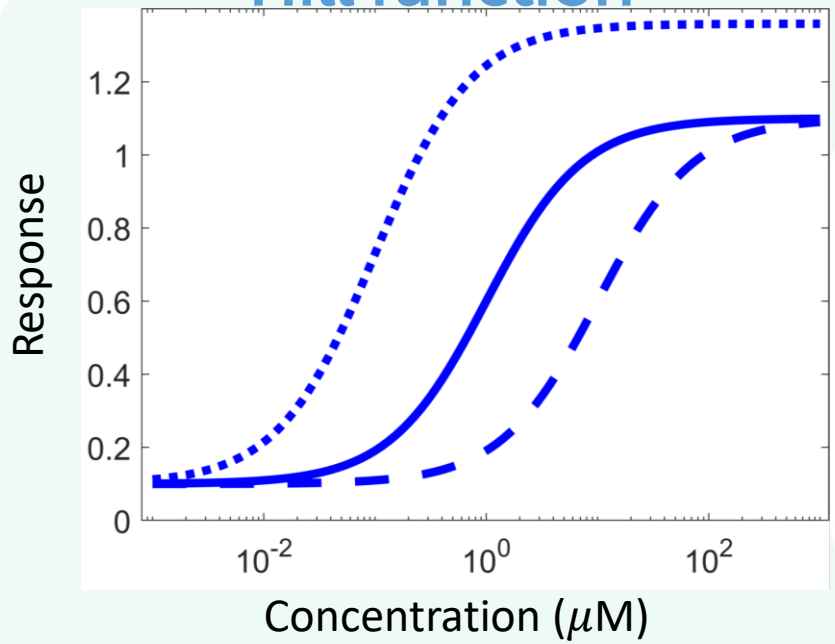
Using Bayesian models to quantify uncertainty

Example dose response data



Hill function



Develop

- Hill equation:

$$f(x|C,\theta,V_{max}) = V_{max}\,\frac{x}{x+\theta} + C$$

- (full Hill equation has exponent on x and θ to obtain sharper curves)

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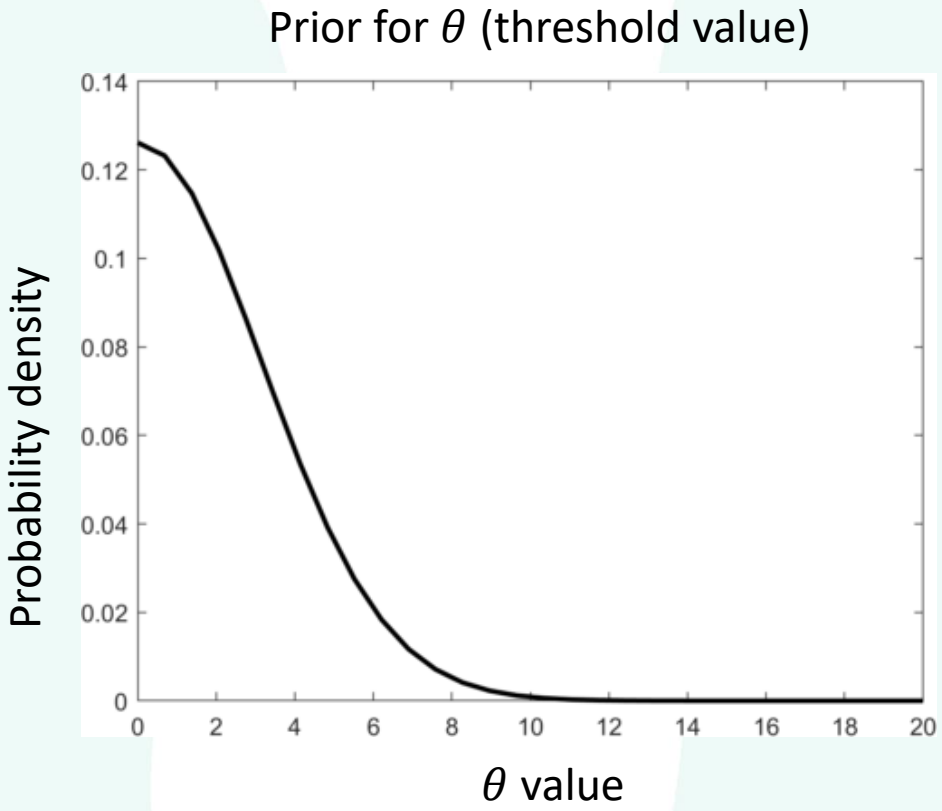
Example of a prior

Develop

- Have parameters θ, C, V_{max} and σ – need to be learned from the data

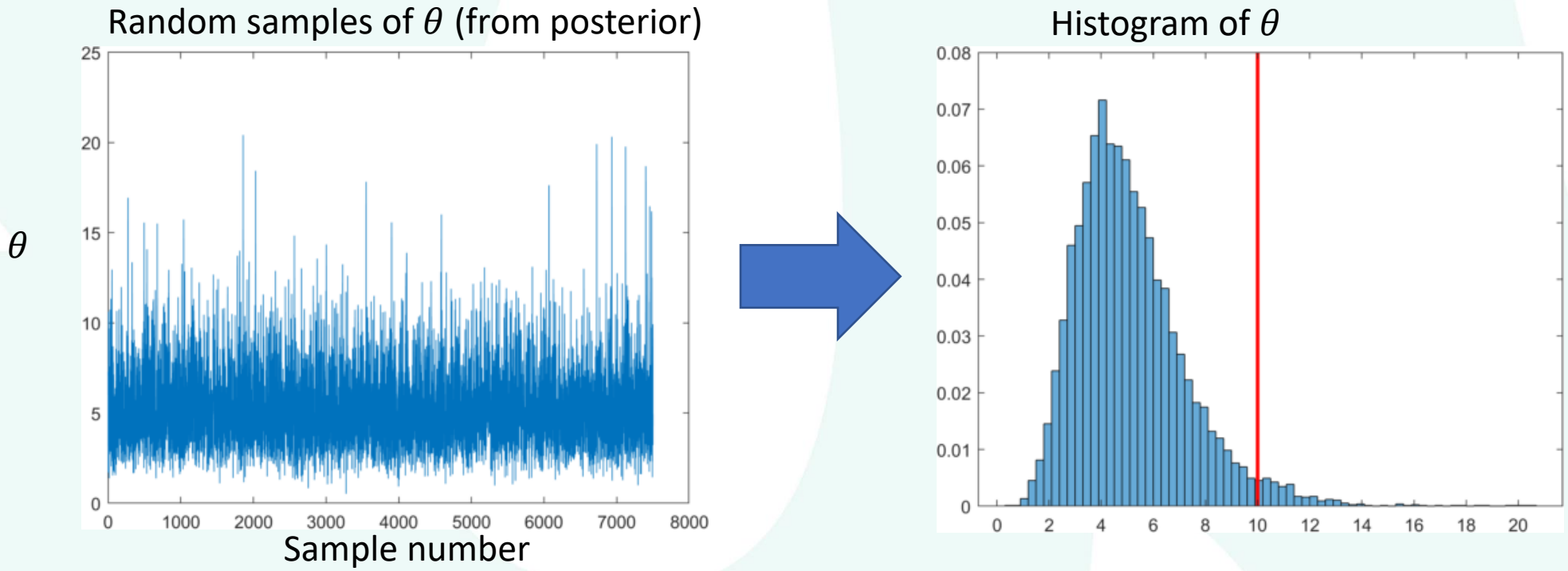


Data

- Typically you only have the measured values that you are fitting to, but you could incorporate prior knowledge (e.g. biologically plausible values) into the prior.

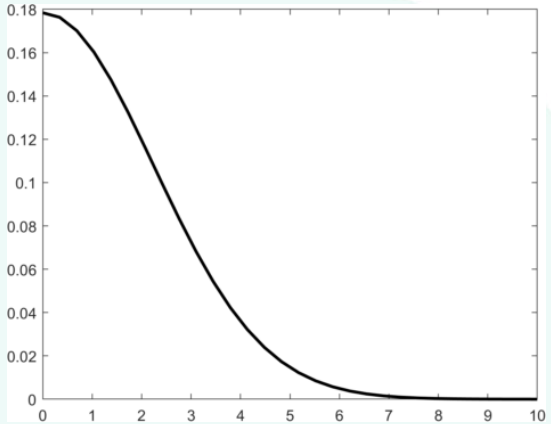
Learning parameters from the data

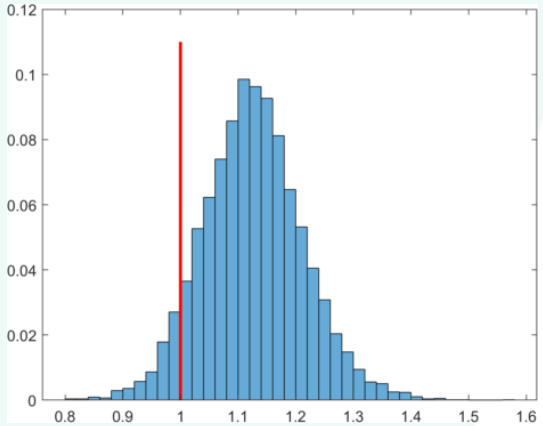
- One things that's important to know about Bayesian statistics is that for most problems, it is impossible to get an exact solution to the posterior.
- Resort to using methods like **Markov Chain Monte Carlo (MCMC)** to take random samples from the distribution.



Learning parameters from the data

Vmax





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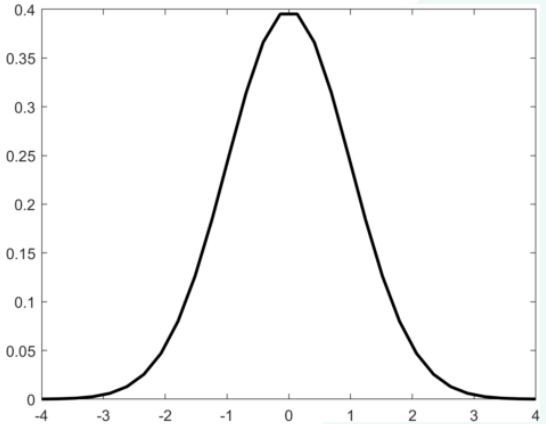

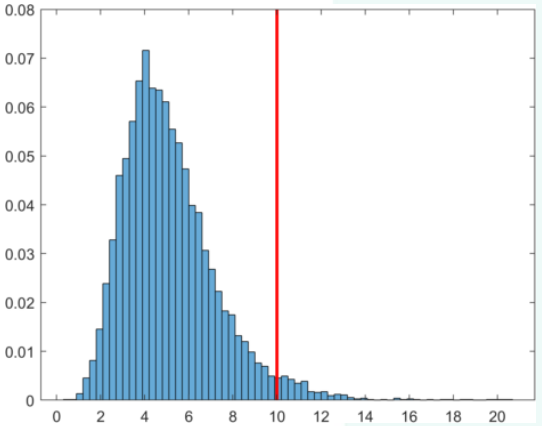

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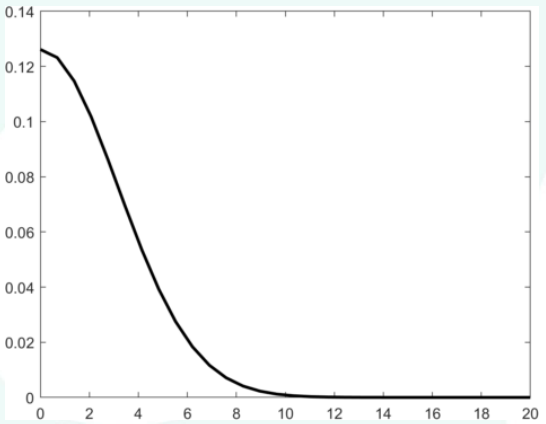
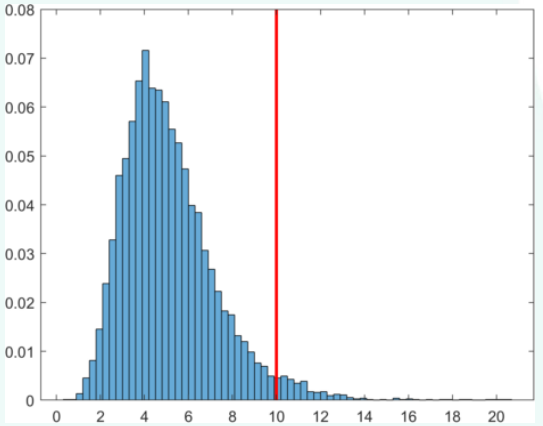
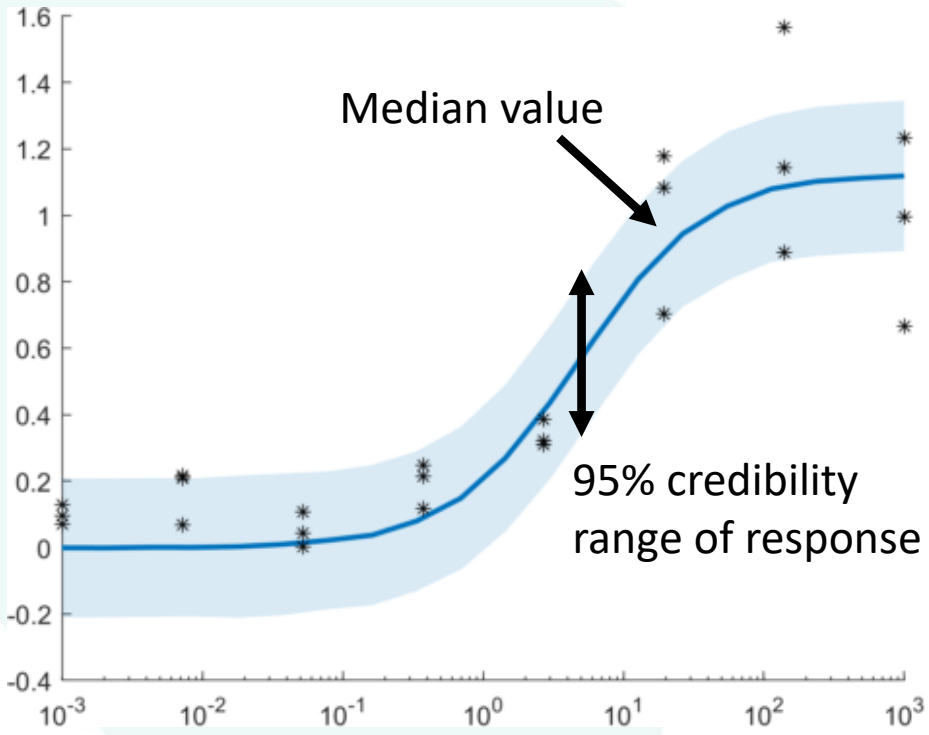
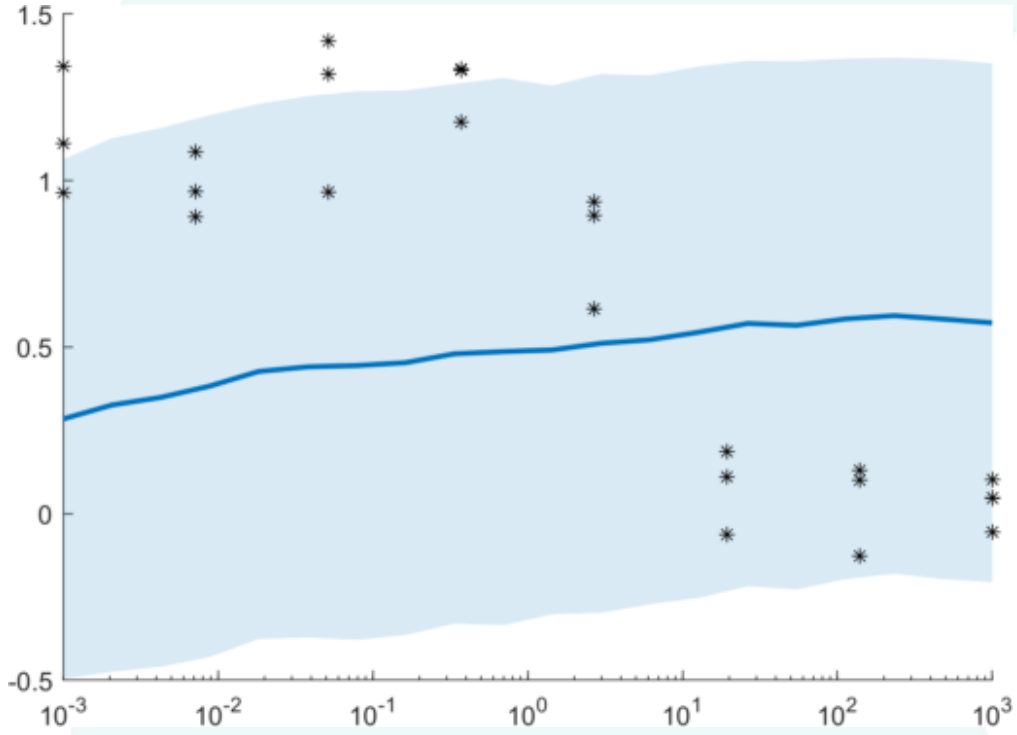




CORRECT LATER ON

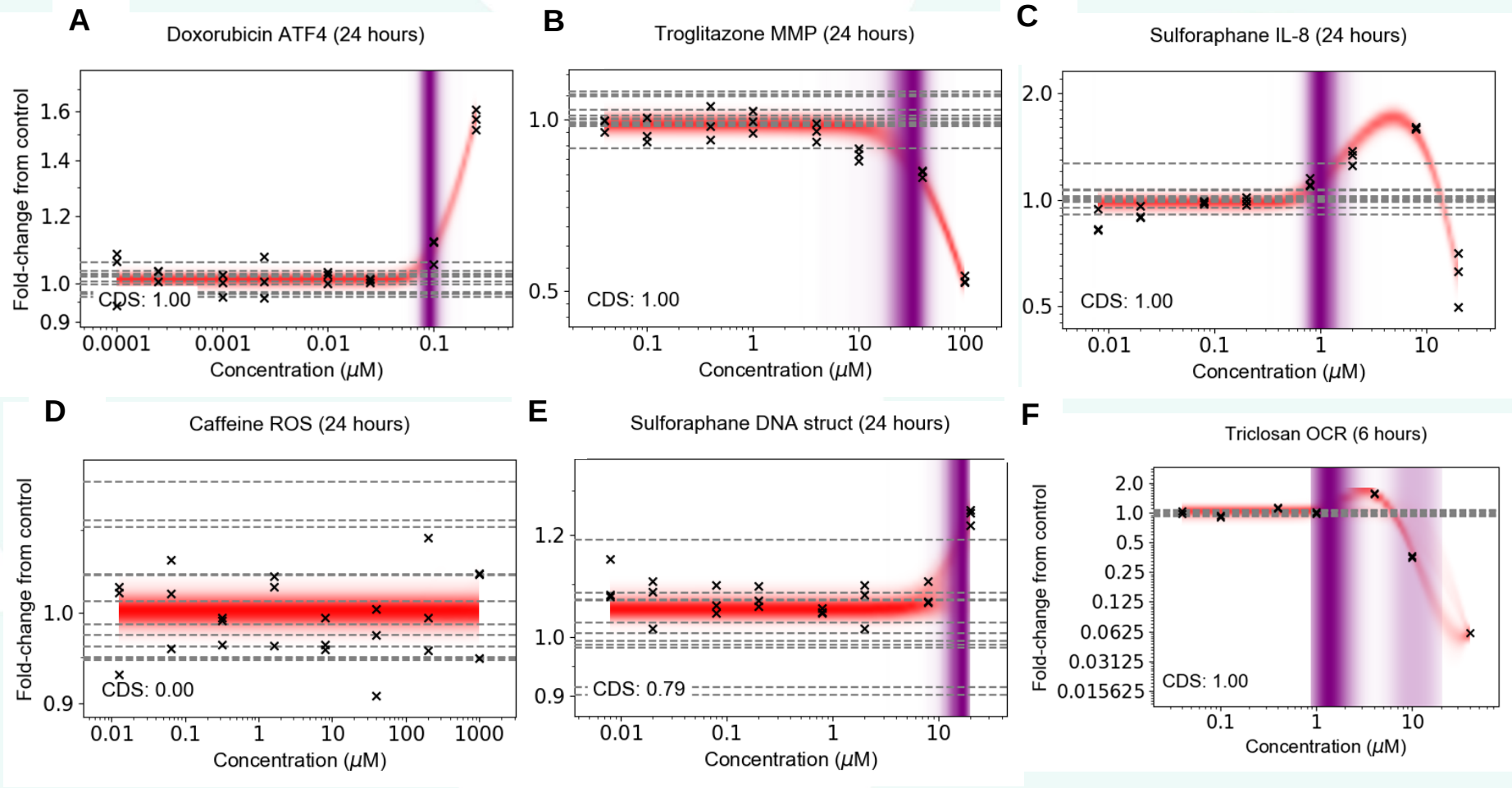
Evaluating the dose response model





- Bayesian models can be evaluated by comparing the predictive distributions to the training data
- When using parametric models is to fit data to multiple models and decide which one is best
- Sometimes you can miss effects, not because there is no effect, but because the model does a poor job of describing the data

Back to the cell stress panel



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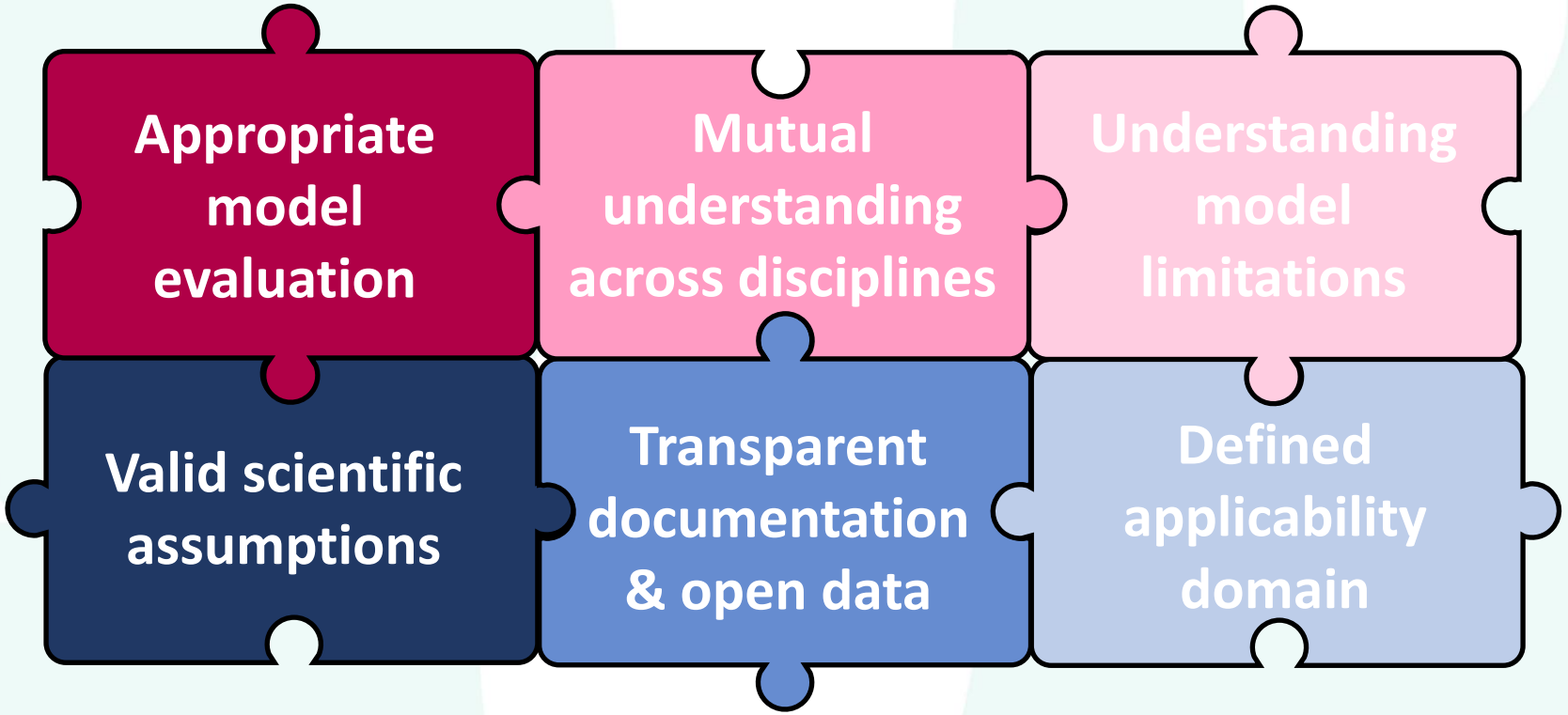
Challenges in the acceptance of using computational approaches in NGRA

What do you think?



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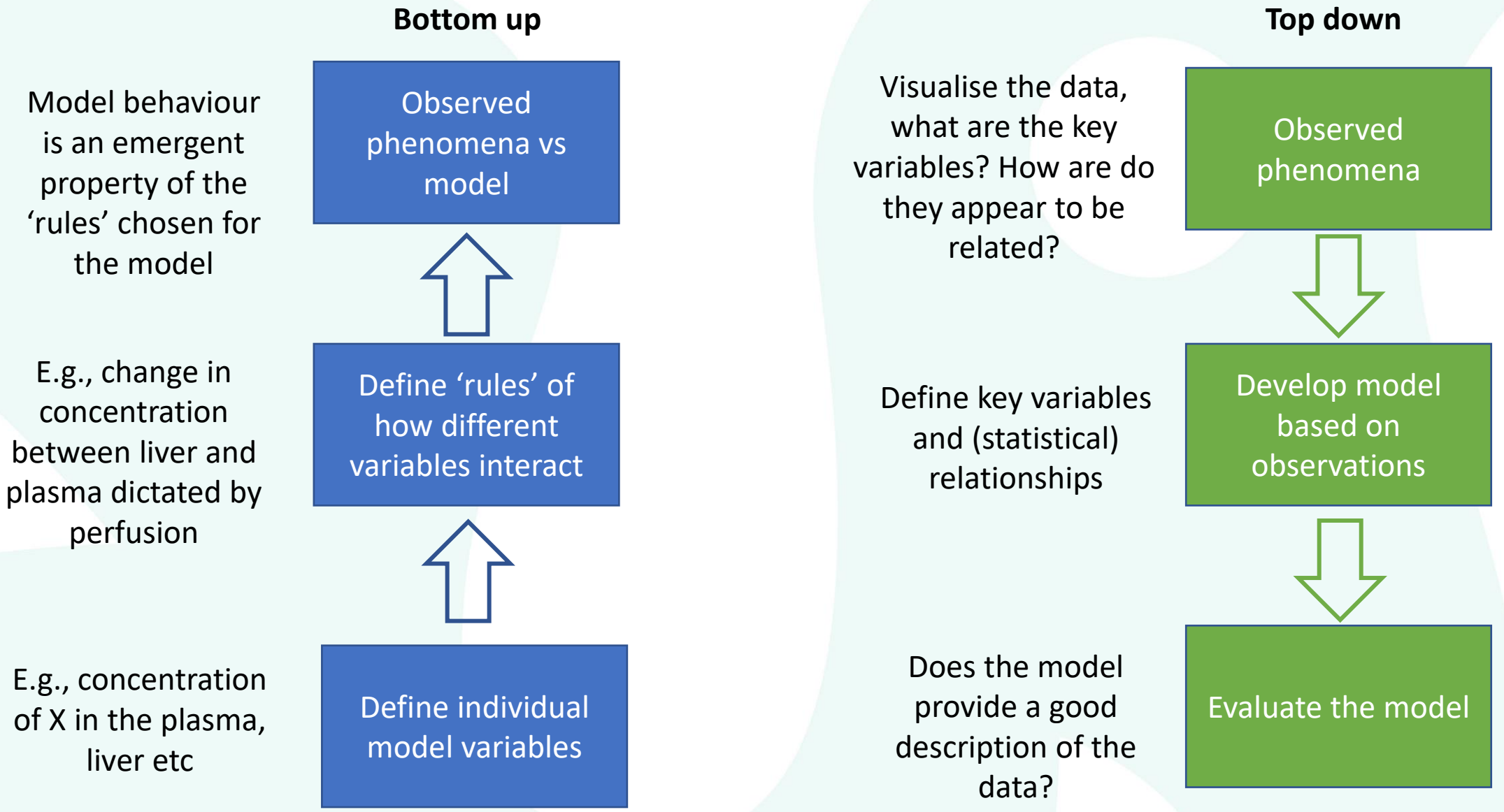
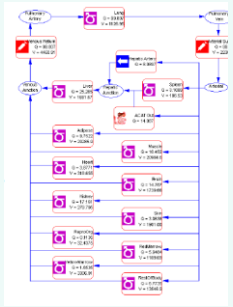
Top down vs bottom modelling



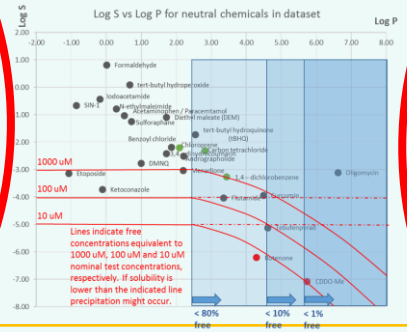
Evaluating a toolbox of NAMs

Back to the toolbox

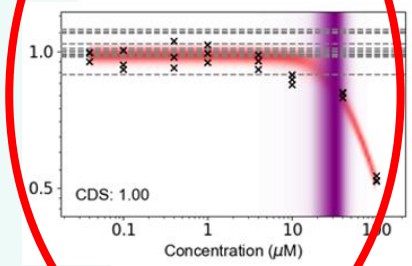
PBK models



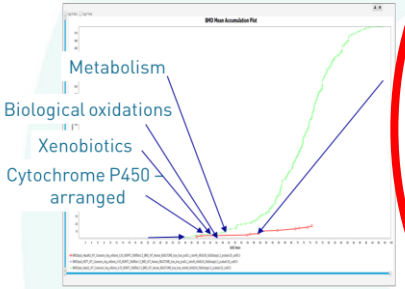
Free concentration



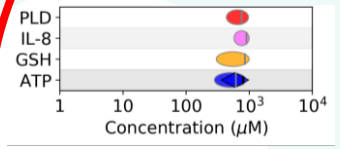
Conc. Resp. models



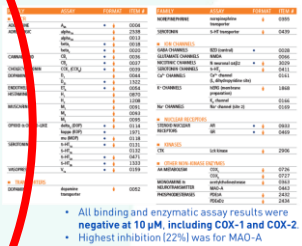
HTTr



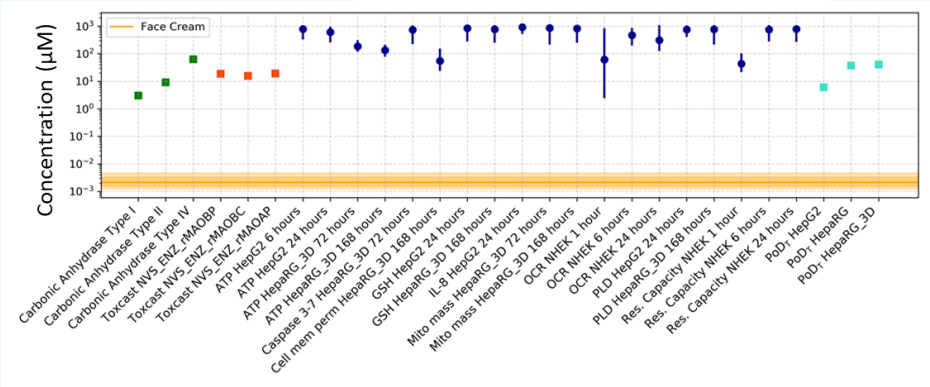
CSP



IPP



Bioactivity exposure ratio



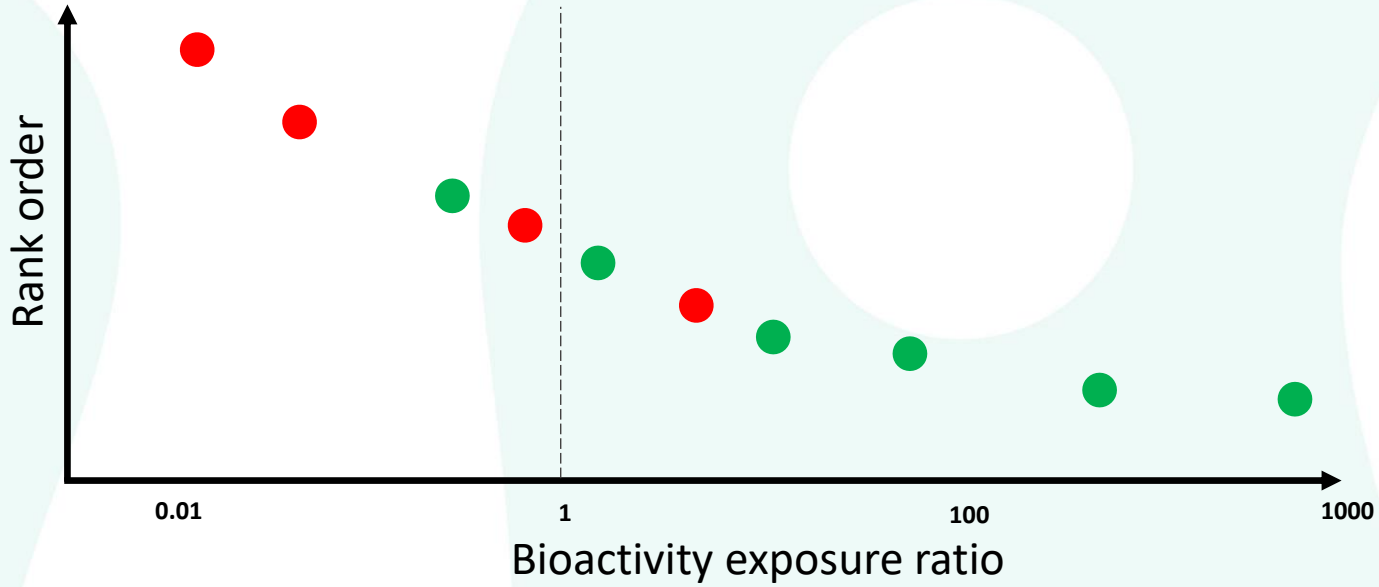
Inform safety decision

HTTr: High-throughput transcriptomics **CSP:** Cell Stress Panel **IPP:** In vitro pharmacological profiling

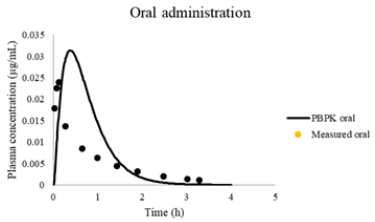
An evaluation strategy for the toolbox

Chemical exposures scenarios

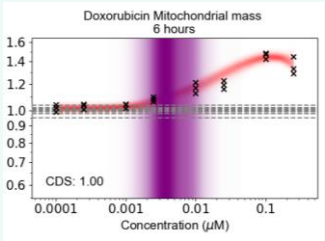
- ‘Low’ risk (from consumer goods perspective) – e.g. foods, cosmetics
- ‘High’ risk (from consumer goods perspective) – e.g. drugs



Define typical use-case scenarios benchmark chemical-exposures; Mixture of High and low risk



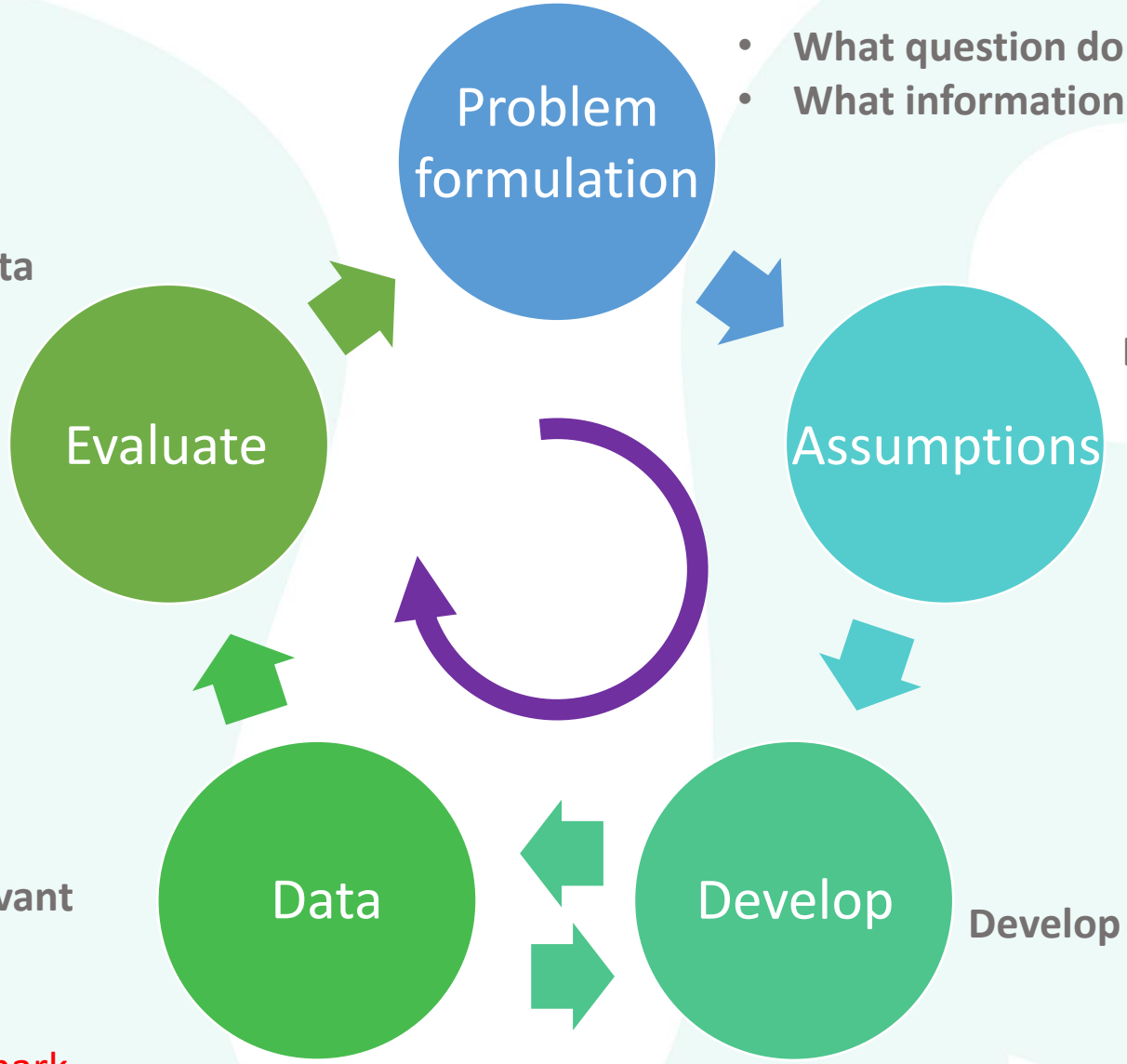
PBK models of systemic exposure



In-vitro cell assays, estimate PoDs

Calculate the bioactivity exposure ratio

Thinking about it in terms of model development



- What question do you want to answer?
- What information do you have available?

Can we use the BERs so that we are protective of human health?

Define model assumptions

The BER can be estimated in terms of the PODs and Cmax from the PBK models

Develop and implement the model

Generate/curate relevant data

Curate relevant benchmark exposures and generate data

How does the model perform?
Does it describe the data well?

Decide on a way to assess how well the toolbox performs

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Identifying suitable benchmarks for the evaluation

Chemical	Exposure scenario	Risk classification
Oxybenzone	**2 scenarios:** 0.5%; 2% sunscreen	Low risk
Caffeine	**2 scenarios:** 0.2% shampoo & coffee oral consumption 50 mg	Low risk
Caffeine	10g – fatal case reports	High risk
Coumarin	**3 scenarios:** 4 mg/d oral consumption; 1.6% body lotion (dermal); TDI 0.1 mg/kg oral	Low risk
Coumarin	400 mg/kg clinical trial ~ 14 months	High risk
Hexylresorcinol	**3 scenarios:** Food residues (3.3 ug/kg); 0.4% face cream; throat lozenge 2.4 mg	Low risk
BHT	Body lotion 0.5%	Low risk
Sulforaphane	**2 scenarios:** Tablet 60 mg/day; food 4.1-9.2 mg/day	Low risk
Niacinamide	**4 scenarios:** oral 12.5-22 mg/kg; dermal 3% body lotion and 0.1 % hair condition	Low risk
Thalidomide	**3 scenarios:** oral tablet 50 mg, 100 mg, 400 mg	High risk
Doxorubicin	75 mg/m2 IV bolus 10 min; 21 days cycles; 8 cycles	High risk
Rosiglitazone	8 mg oral tablet	High risk
Valproic Acid (VPA)	**2 scenarios:** oral tablet 1000 mg & > 60 mg/kg	High risk
Paraquat	Accidental ingestion 35 mg/kg	High risk

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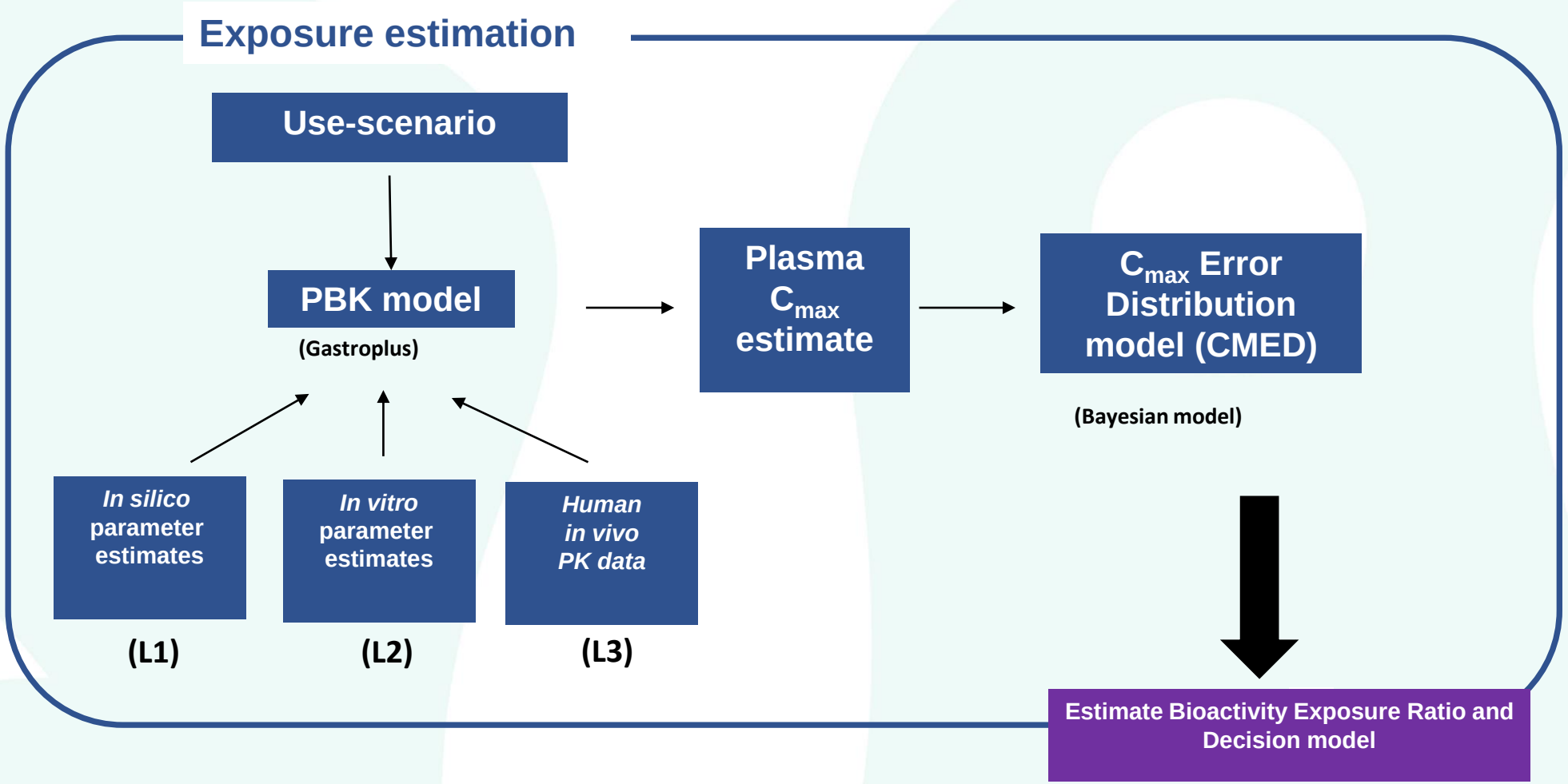
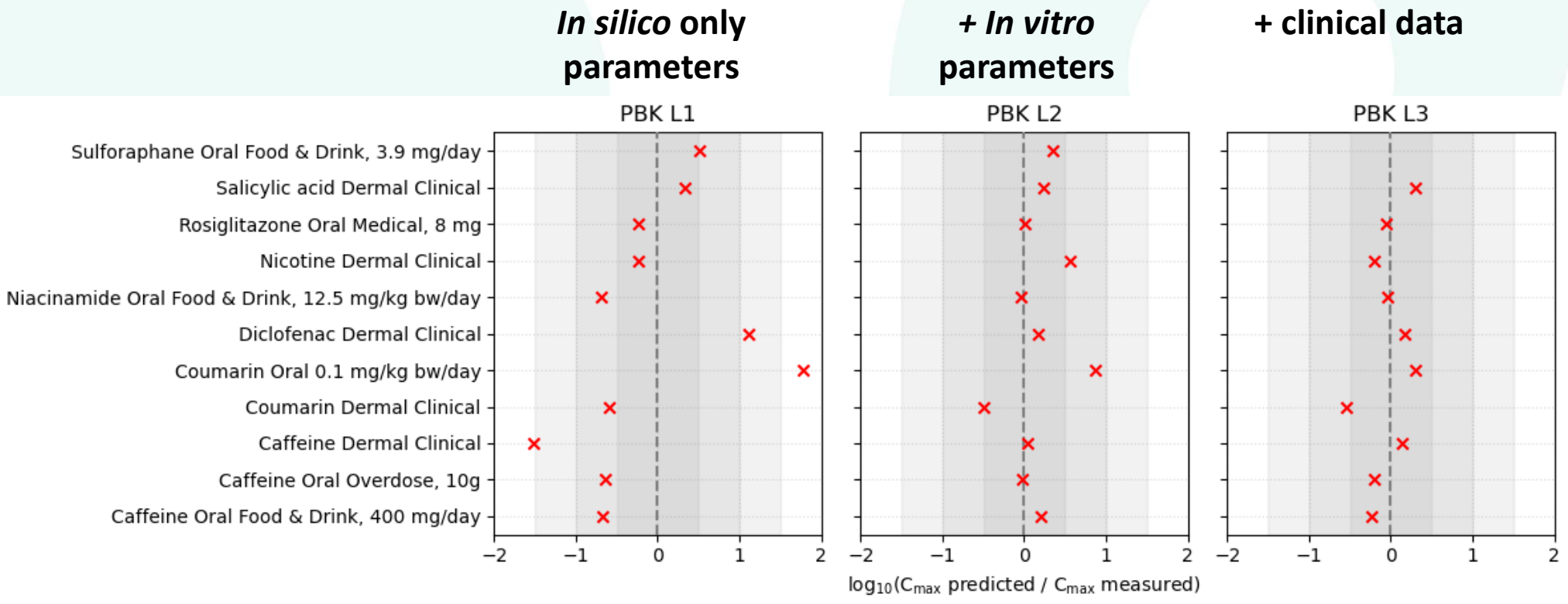
Using PBK models to predict Cmax



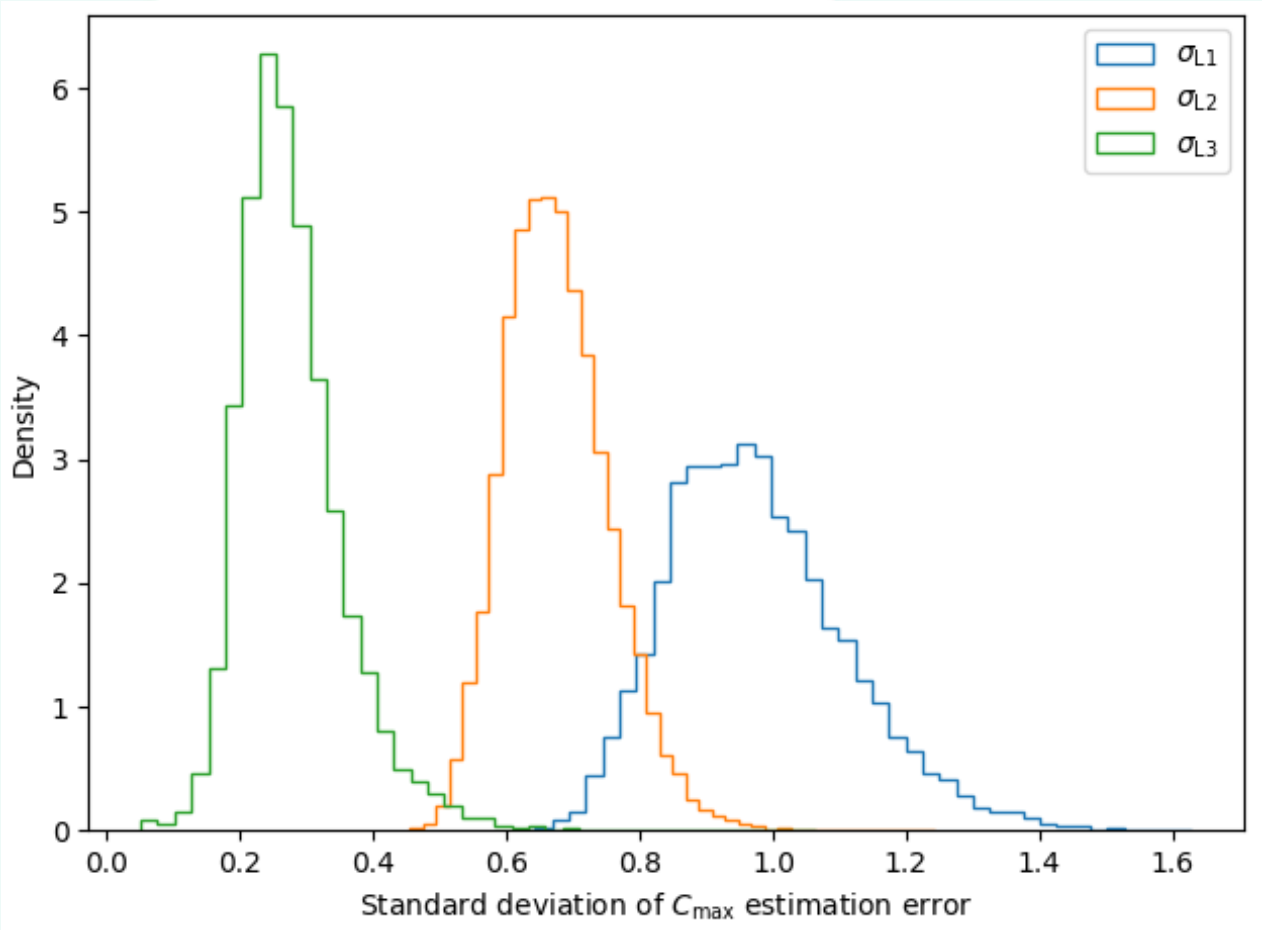
- Used a (**bottom-up**) PBK model to predict Cmax under different parameterisations
- Used a (**top down**) Bayesian statistical model to quantify the potential error in the est

Quantifying the error in the Cmax estimates

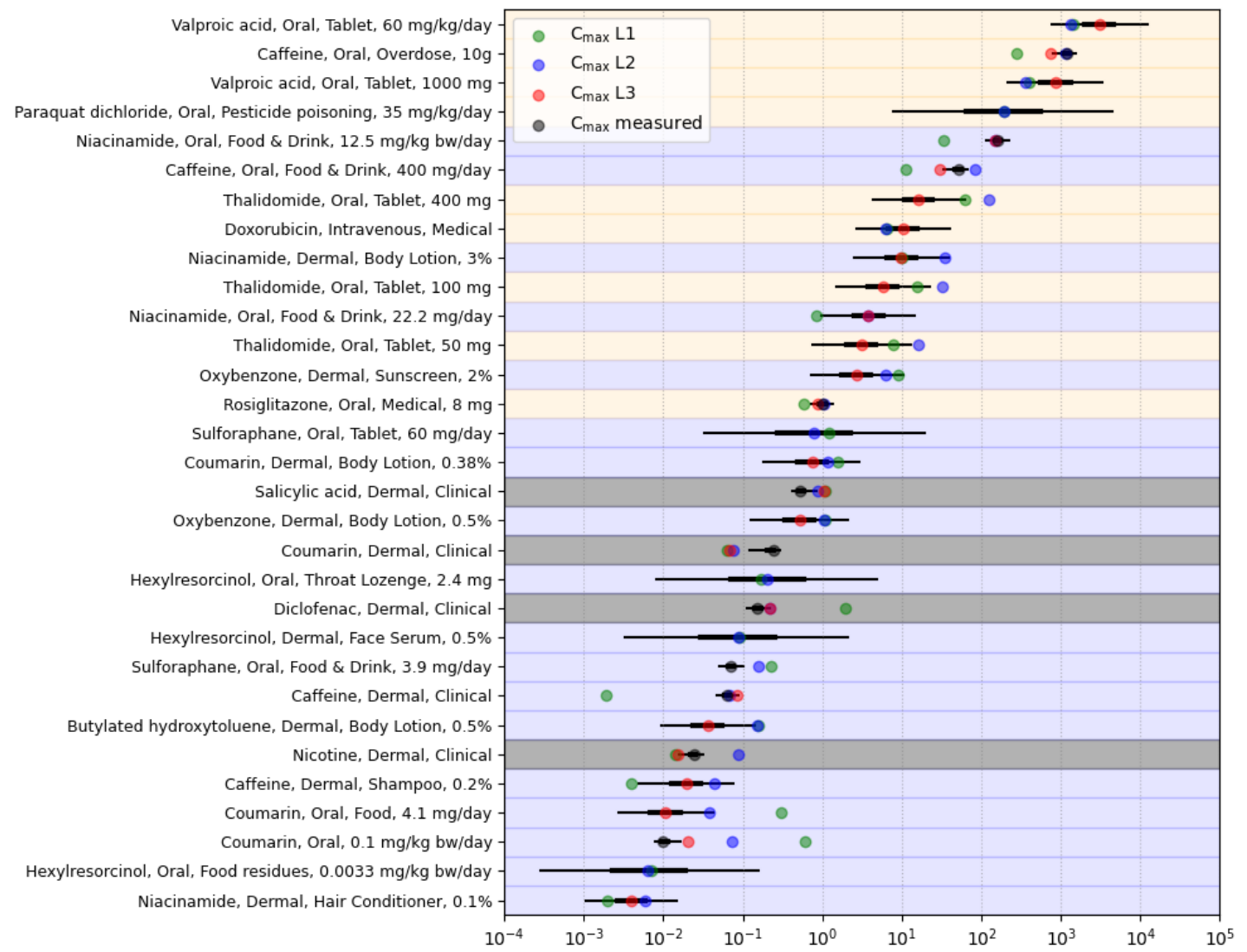


- The PBK prediction error decreases as we go through the different parameterisation levels
- This is an empirical observation

Using a Bayesian model to learn the prediction error

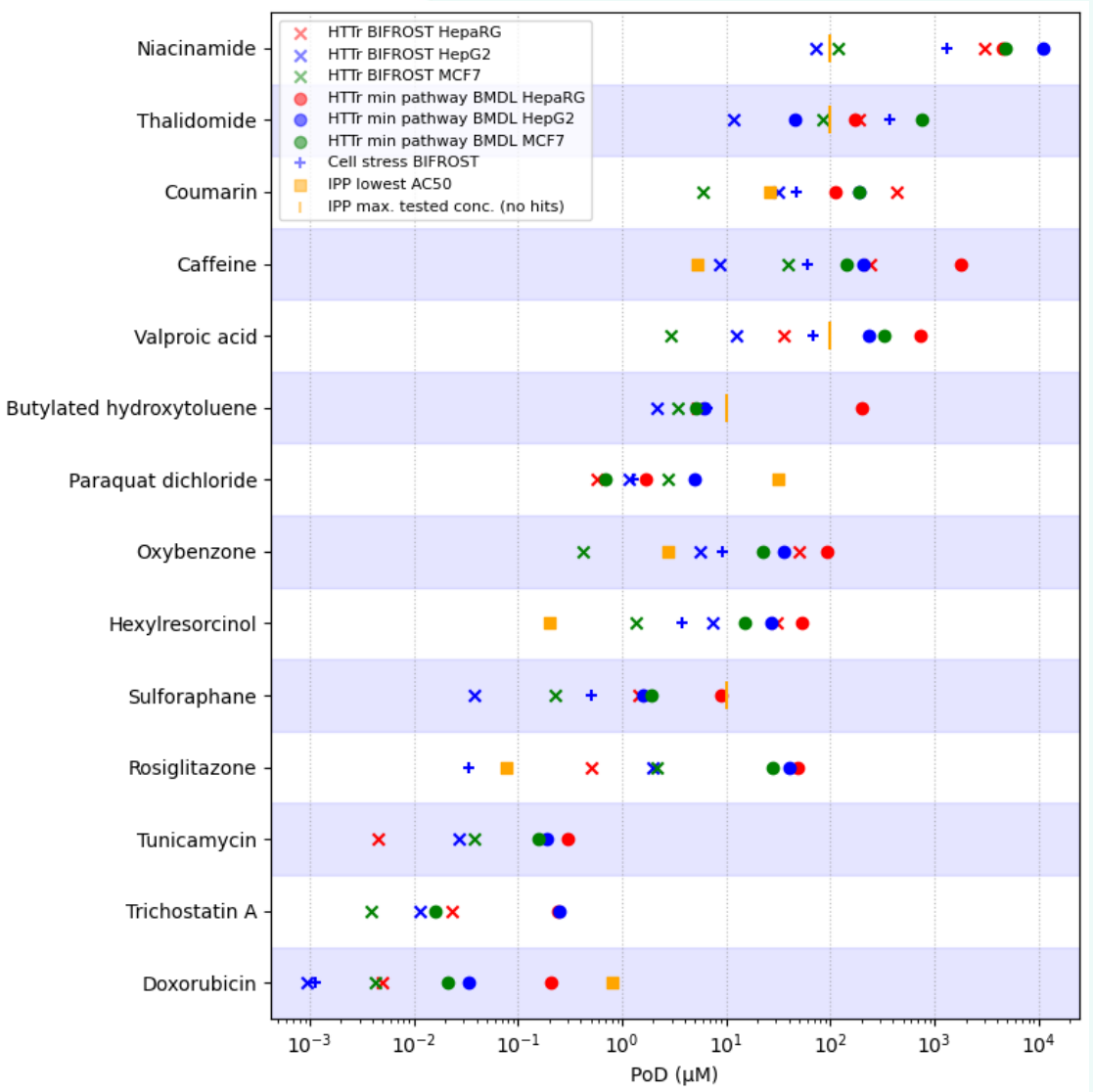


Using PBK models to predict Cmax

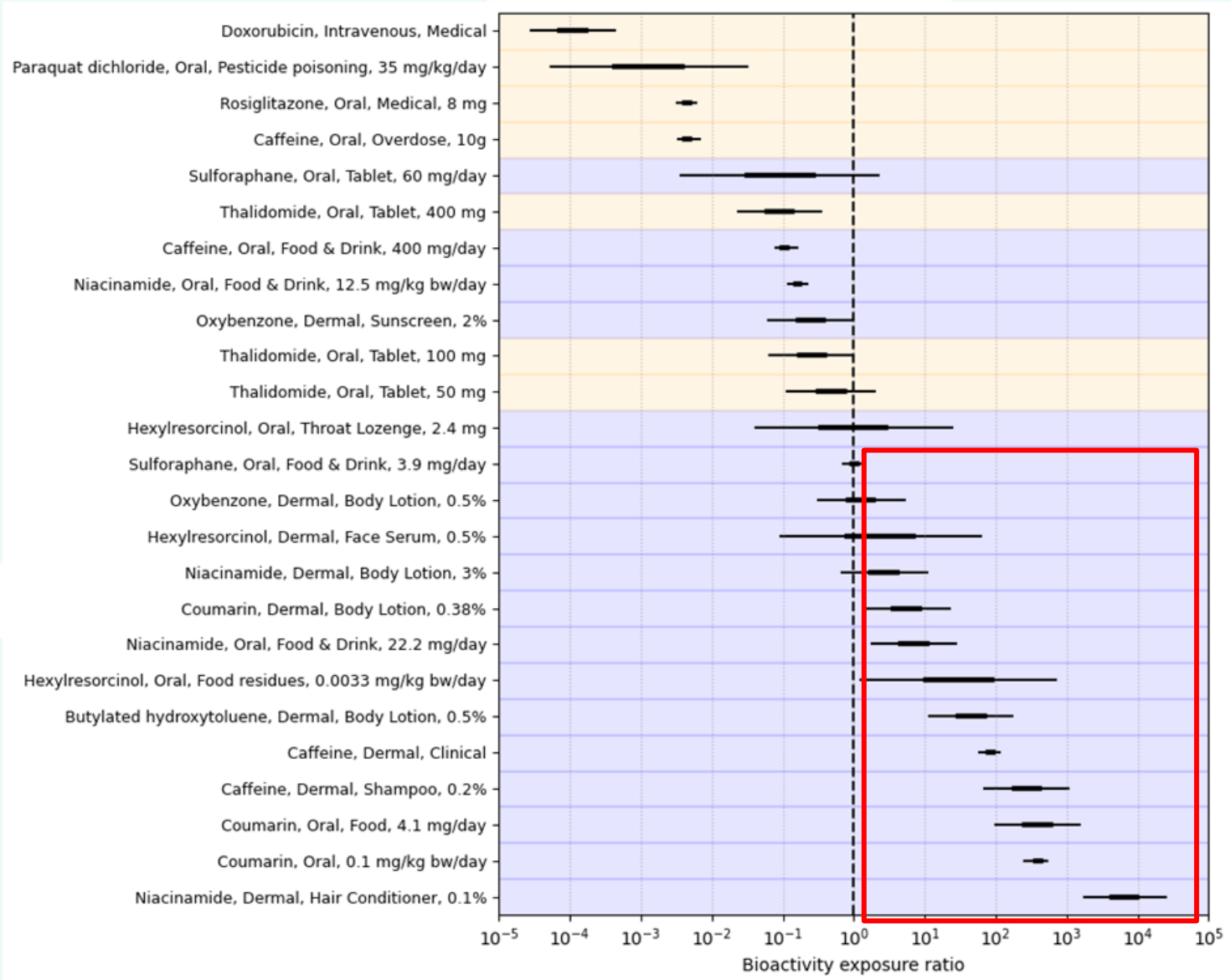


PODS from the bioactivity platforms

Dose response plots



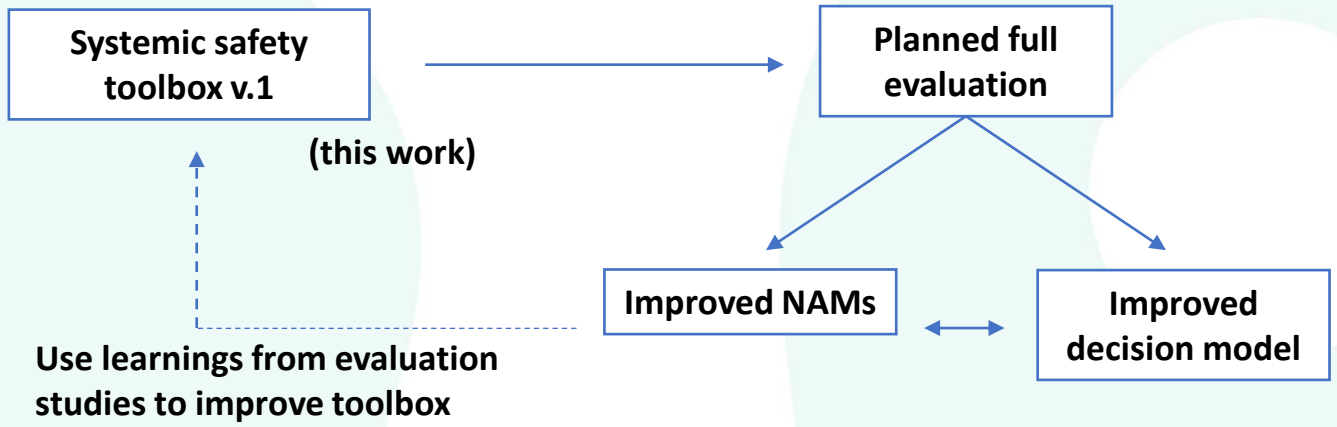
Initial results indicate the toolbox is protective



- Blue: low risk chemical-exposure scenario
- Yellow: high risk chemical-exposure scenario

- Protectiveness: 100%
- Utility: 62%

Next step for the toolbox – the full evaluation



- Planning to extend evaluation to ~40 chemicals with ~60 associated **high risk** and **low risk** exposure scenarios.
- Also in collaboration with US-EPA, expanding range of NAMs
- Adopt **iterative approach** to evaluating and then identifying potential improvements to the toolbox.
- Use of concepts from used model evaluation and development should help build confidence in the approach.

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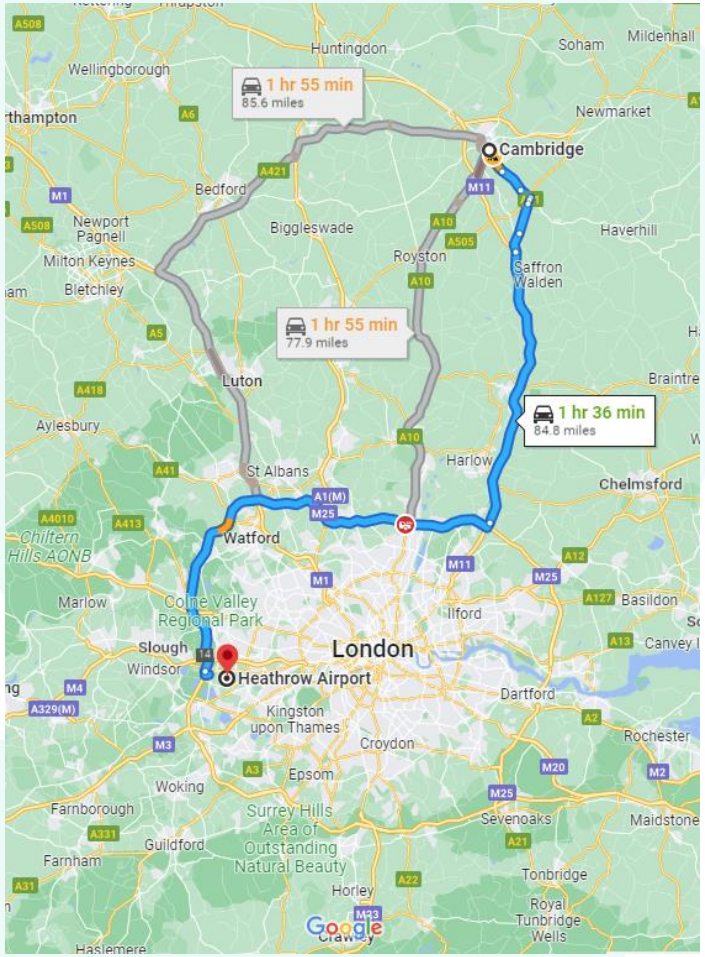
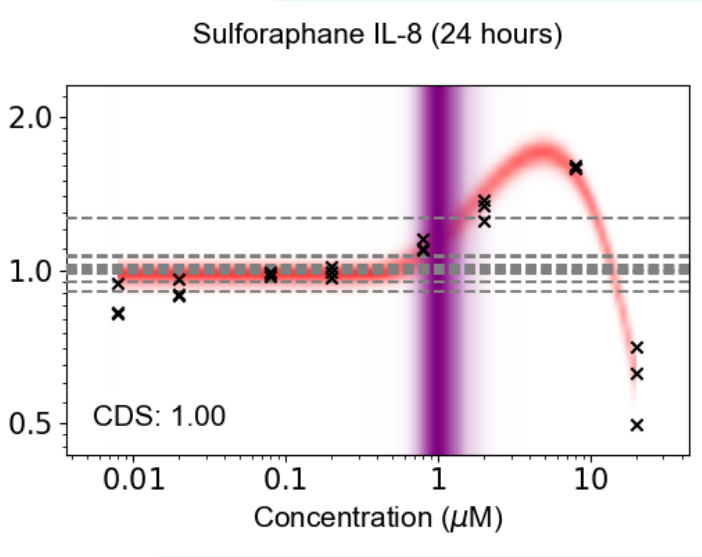
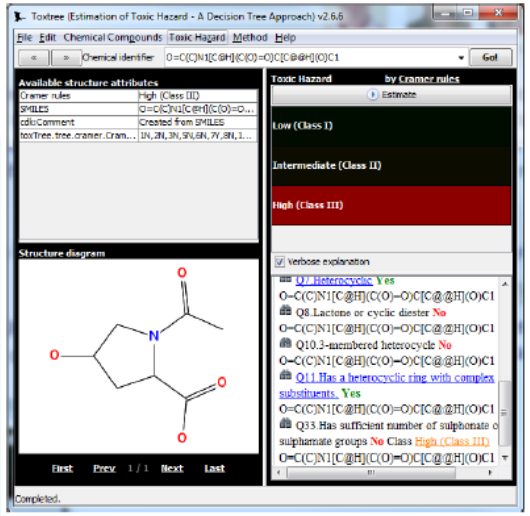
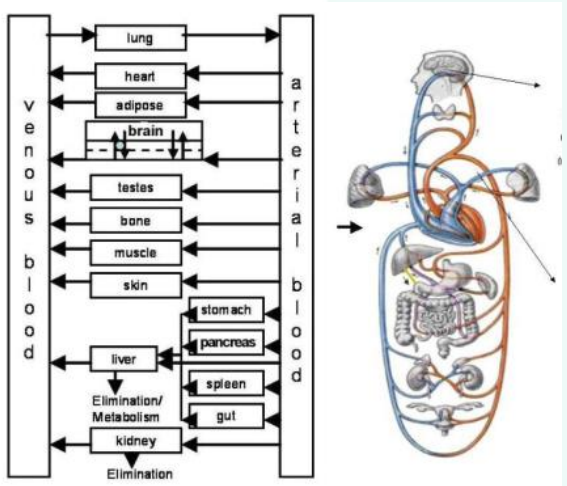
Thinking about the future...







ToxTree



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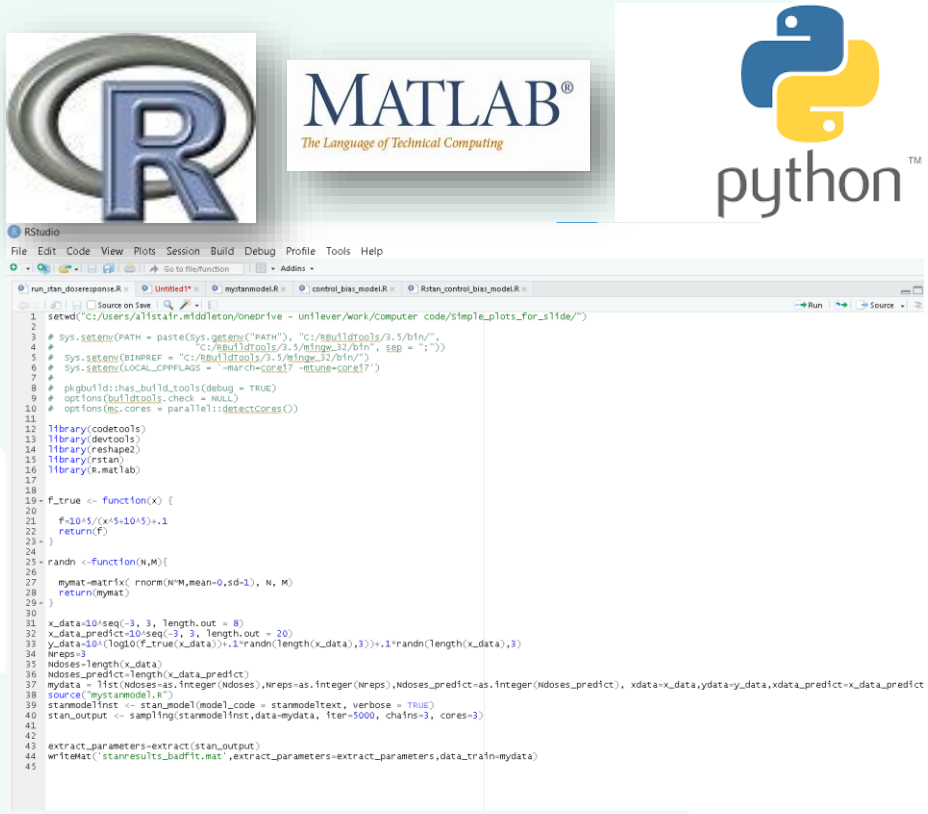
Getting started with computational approaches...

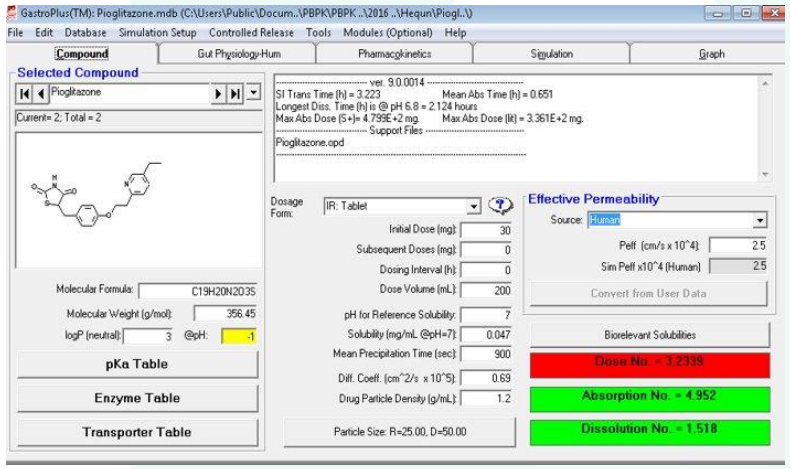
Learning to code vs using existing tools

Programming



Graphical user interfaces

PBK software



SIMCYP real solutions from virtual populations

GastroPlus™

BERKELEY MADONNA Modeling and Analysis of Dynamic Systems

Bayer Technology Services PK-SIM®

Dose response software

tcpl 2.0
Data Processing
National Center for Computational Toxicology, US EPA

https://cran.r-project.org/web/packages/tcpl/vignettes/Data_processing.html

BBMD™ BAYESIAN BENCHMARK DOSE MODELING SYSTEM

https://benchmarkdose.com/

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