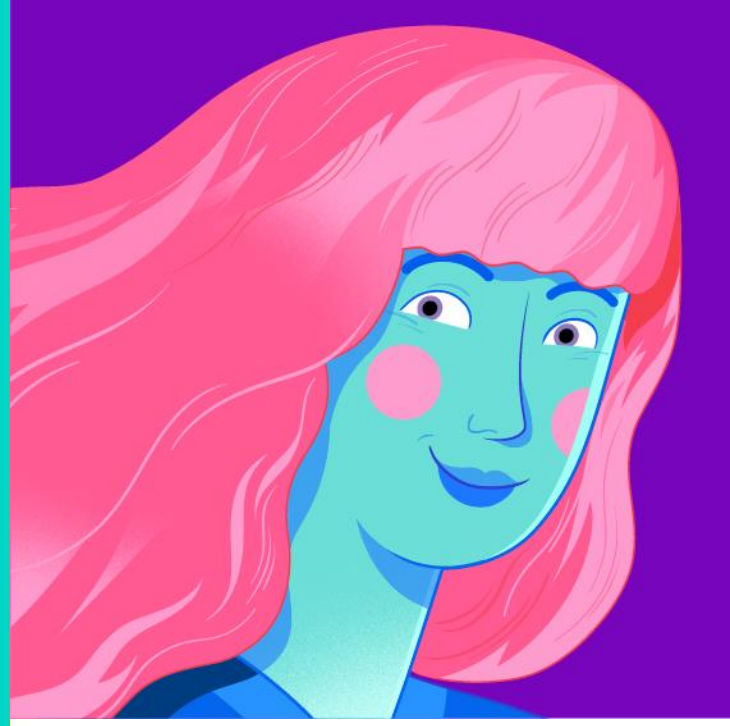
Decision making in Next Generation Risk Assessment (NGRA)



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30/05/2022

Learning objectives

- Give an overview of next generation risk assessment.
- Awareness of different computational approaches that are used (e.g., Bayesian inference, dose response 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).

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

Web Resource

Unilever's Safety and Environmental Assurance Centre'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**

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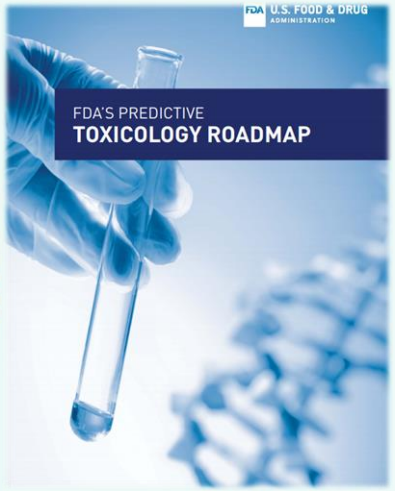
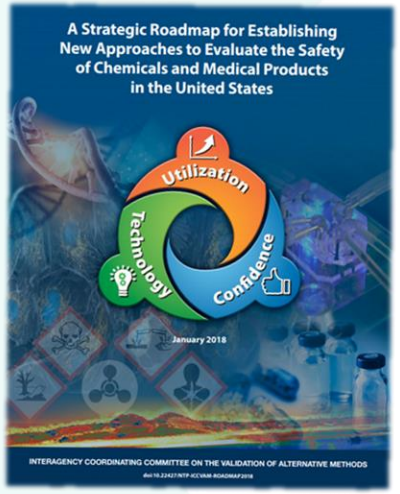
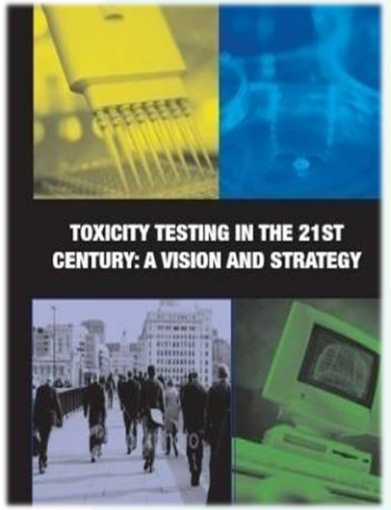
Next Generation Risk Assessment (NGRA)

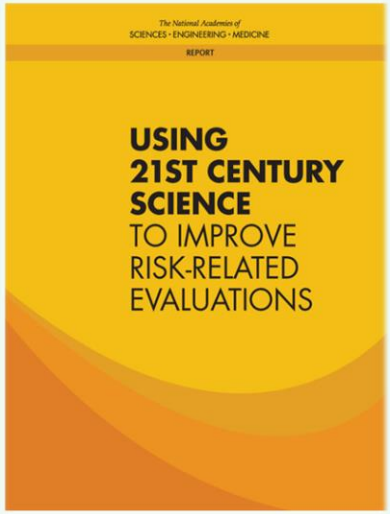
*NGRA is defined as **an exposure-led**, **hypothesis-driven** risk assessment approach that **integrates New Approach Methodologies (NAMs)** to assure **safety without the use of animal testing***

Safety without animal testing









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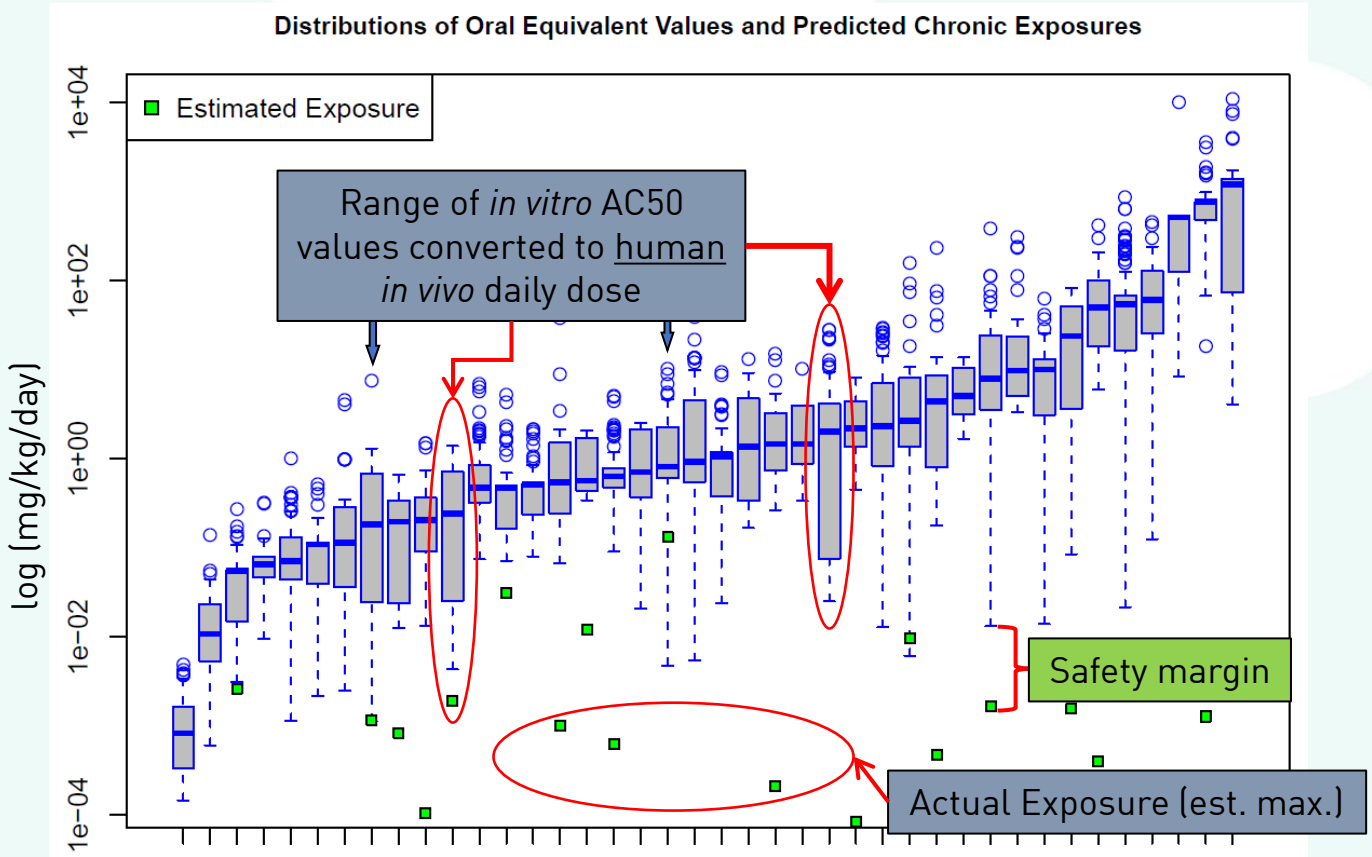
In Vitro Bioactivity vs Bioavailabilty



Hepatic clearance and plasma protein binding determinations

"Protection not Prediction"

Slide from Dr Rusty Thomas, EPA, with thanks

Rotroff, *et al.* Tox.Sci 2010

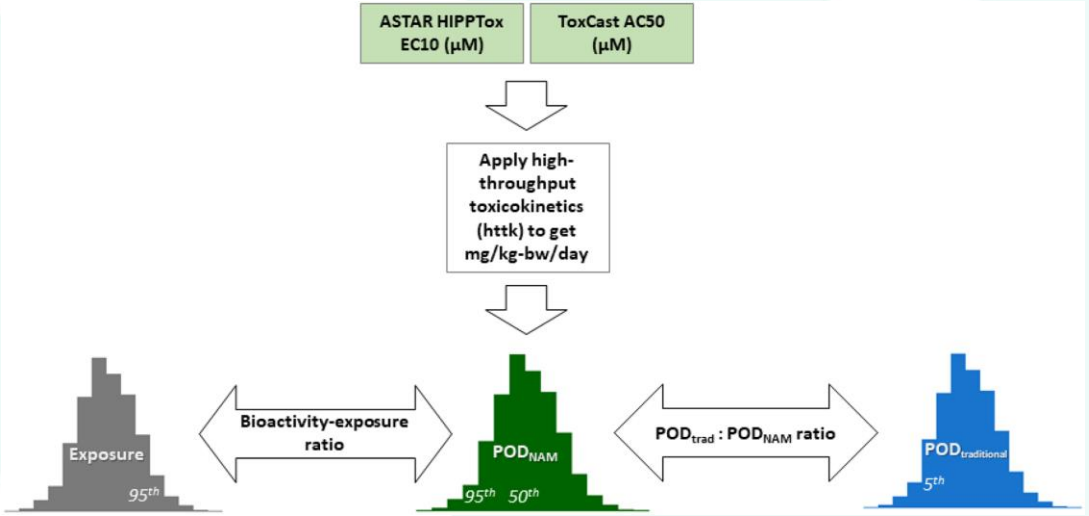
Integration of exposure and bioactivity for decision-making – Example from the *Accelerating the Pace of Chemical Risk Assessment* (APCRA) initiative

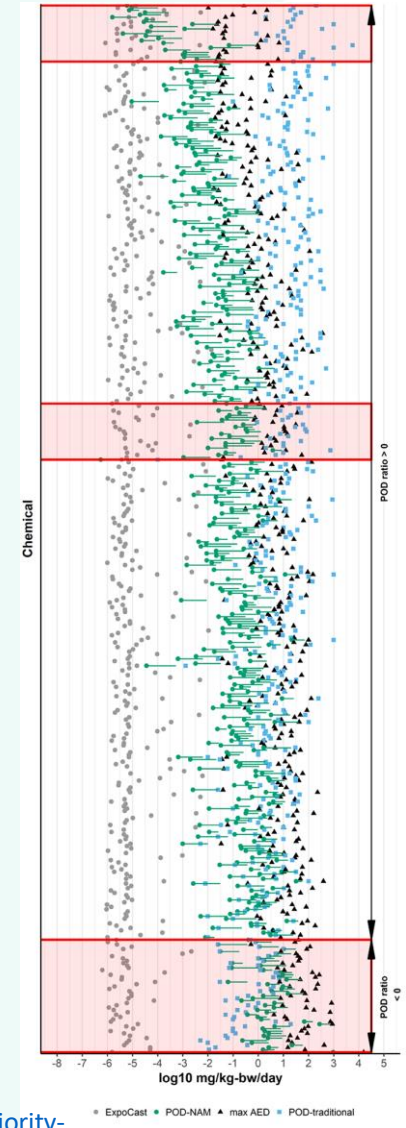
OXFORD SOT Society of Toxicology academic.oup.com/toxsci Tox Spotlight article

TOXICOLOGICAL SCIENCES, 173(1), 2020, 202–225
doi: 10.1093/toxsci/kfz201
Advance Access Publication Date: September 18, 2019
Research Article

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

Katie Paul Friedman ,*,[1] Matthew Gagne,[†] Lit-Hsin Loo,[‡] Panagiotis Karamertzanis,[§] Tatiana Netzeva,[§] Tomasz Sobanski,[§] Jill A. Franzosa,[¶] Ann M. Richard,[*] Ryan R. Lougee,[*,||] Andrea Gissi,[§] Jia-Ying Joey Lee,[‡] Michelle Angrish,[|||] Jean Lou Dorne,[||||] Stiven Foster,[#] Kathleen Raffaele,[#] Tina Bahadori,[||] Maureen R. Gwinn,[*] Jason Lambert,[*] Maurice Whelan,[**] Mike Rasenberg,[§] Tara Barton-Maclaren,[†] and Russell S. Thomas *





- Of the 448 substances, 89% had a $POD_{NAM,95}$ that was less than the traditional POD ($POD_{traditional}$) value.
- Bioactivity:exposure ratios (BERs), useful for identification of substances with potential priority, demonstrated that high-throughput exposure predictions were greater than the PODNAM,95 for 11 substances.

https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/science-approach-document-bioactivity-exposure-ratio-application-priority-setting-risk-assessment.html

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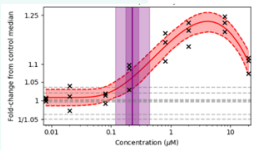
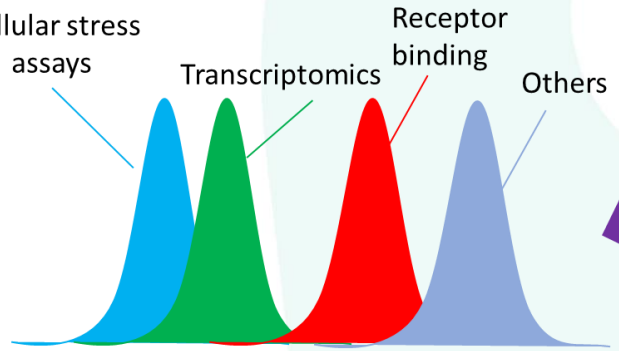
Integration of exposure and bioactivity for decision-making – The assessment is designed to prevent harm

Hazard identification and characterisation of ingredients

Point of departure derived from concentration-response data

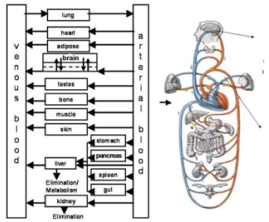




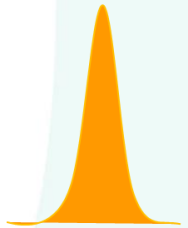
Consumer Exposure characterisation

Skin pen

Exposure models (PBK, free/total concentration)



Exposure estimation: Plasma C_{max}

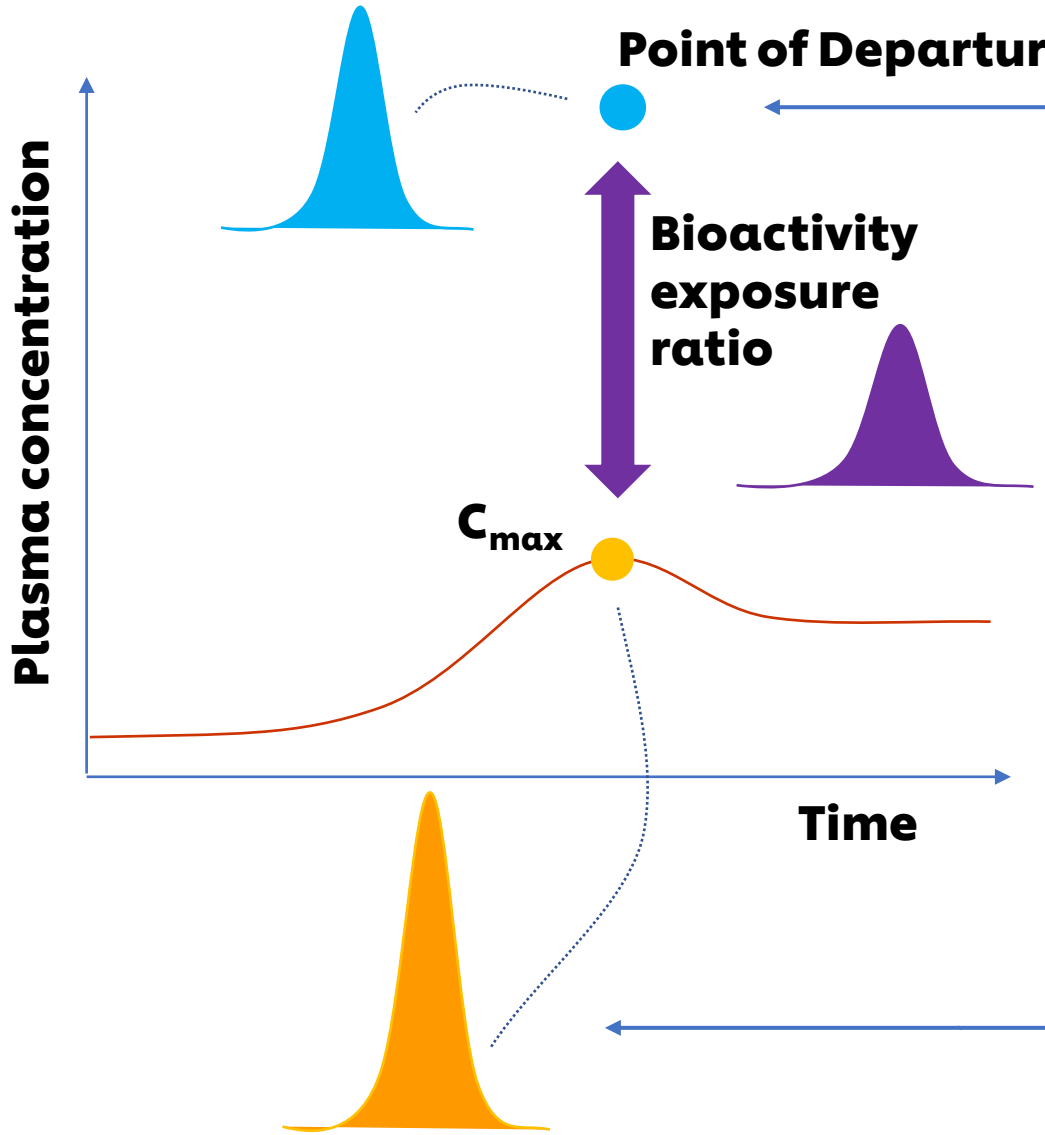


Risk Assessment

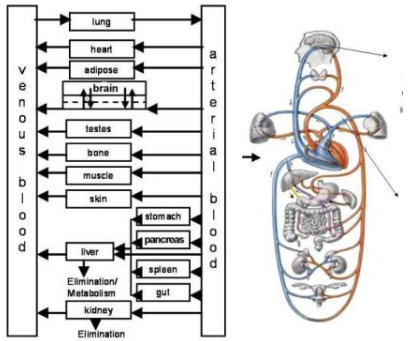
Calculation of Bioactivity Exposure Ratio (BER)

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

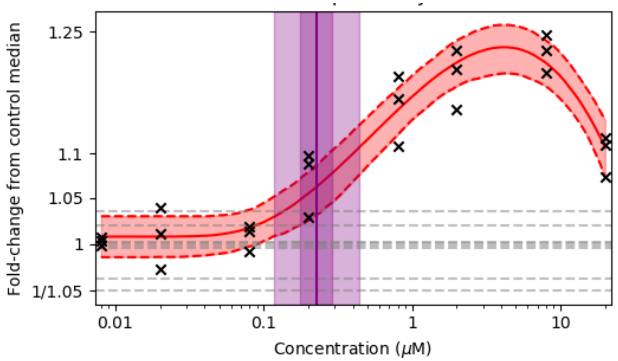
Bioactivity Exposure Ratio



Exposure models (PBK, free/total concentration)



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



*NAM = New Approach Methodology

Integration of exposure and bioactivity for decision-making – Case studies

NAMs to support hypothetical read-across NGRA case studies (e.g. caffeine and parabens)

Regulatory Toxicology and Pharmacology 123 (2021) 104931

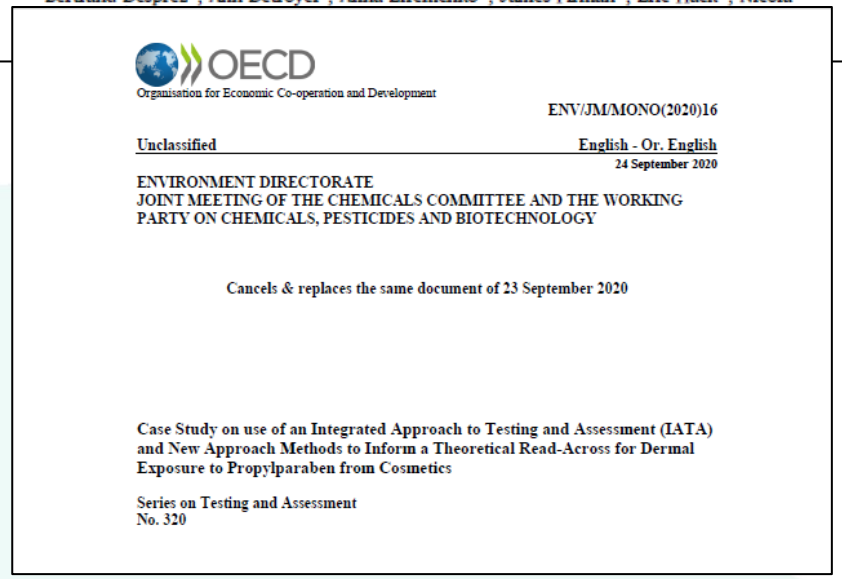
Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

New framework for a non-animal approach adequately assures the safety of cosmetic ingredients – A case study on caffeine

Dagmar Bury [a], Camilla Alexander-White [b], Harvey J. Clewell III [c], Mark Cronin [d], Bertrand Desprez [e], Ann Detroyer [f], Alina Efremenko [g], James Firman [d], Eric Hack [g], Nicola



OECD

Organisation for Economic Co-operation and Development

ENV/JM/MONO(2020)16

Unclassified — English - Or. English

24 September 2020

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

Cancels & replaces the same document of 23 September 2020

Case Study on use of an Integrated Approach to Testing and Assessment (IATA) and New Approach Methods to Inform a Theoretical Read-Across for Dermal Exposure to Propylparaben from Cosmetics

Series on Testing and Assessment
No. 320

NAMs applied in an *ab initio* hypothetical NGRA case study

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

TOXICOLOGICAL SCIENCES, 176(1), 2020, 236–252

doi: 10.1093/toxsci/kfaa048
Advance Access Publication Date: April 10, 2020
Research article

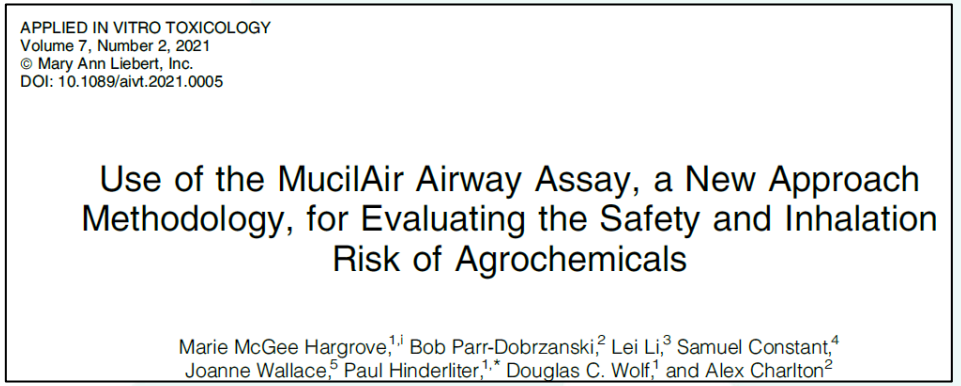
A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products

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

Unilever Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, Bedfordshire MK44 1LQ, UK

[1]To whom correspondence should be addressed. Fax: +44(0)1234 264 744. E-mail: maria.baltazar@unilever.com.

NAMs applied in real-life chemical safety assessments



APPLIED IN VITRO TOXICOLOGY
Volume 7, Number 2, 2021
© Mary Ann Liebert, Inc.
DOI: 10.1089/aivt.2021.0005

Use of the MucilAir Airway Assay, a New Approach Methodology, for Evaluating the Safety and Inhalation Risk of Agrochemicals

Marie McGee Hargrove,[1,i] Bob Parr-Dobrzanski,[2] Lei Li,[3] Samuel Constant,[4] Joanne Wallace,[5] Paul Hinderliter,[1,*] Douglas C. Wolf,[1] and Alex Charlton[2]

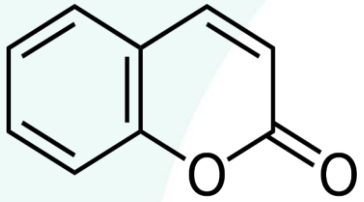
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

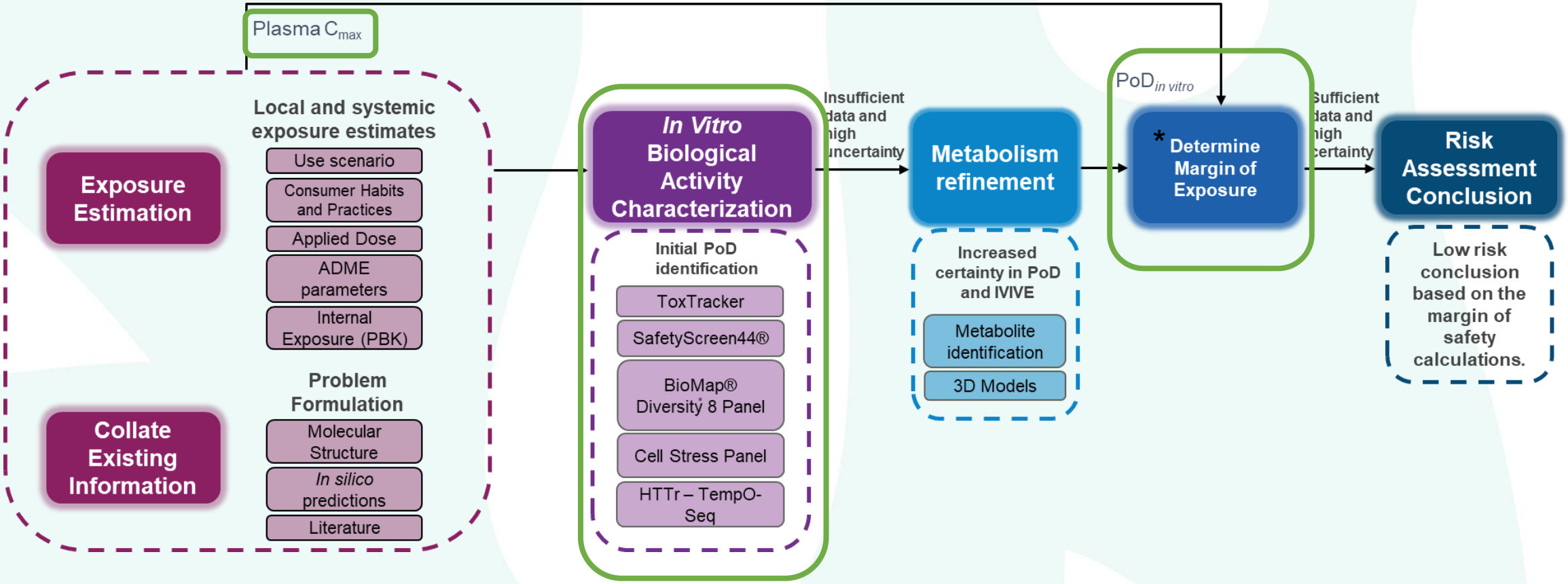
https://www.regulations.gov/document/EPA-HQ-OPP-2011-0840-0080

Unilever

Example how to integrate NAMs for a NGRA: coumarin case study

0.1% COUMARIN IN FACE CREAM AND BODY LOTION (NEW FRAGRANCE)

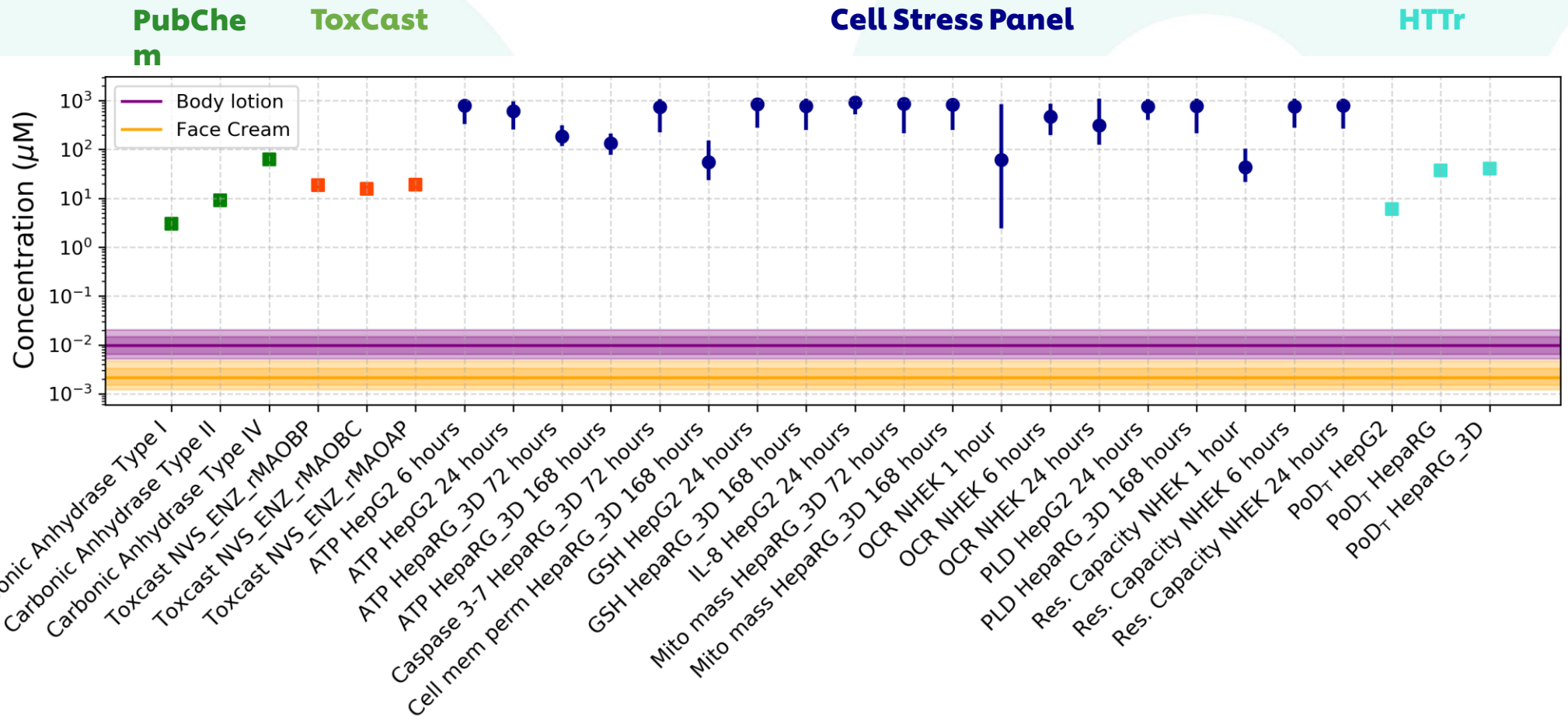




Baltazar *et al.*, (2020) *Tox Sci* Volume 176, Issue 1, 236–252

*or Bioactivity exposure ratio (BER)

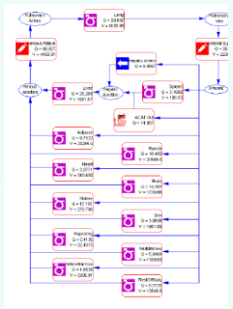
Exposure and PoD are plotted and used to derive a Bioactivity-Exposure Ratio (MoE/BER)



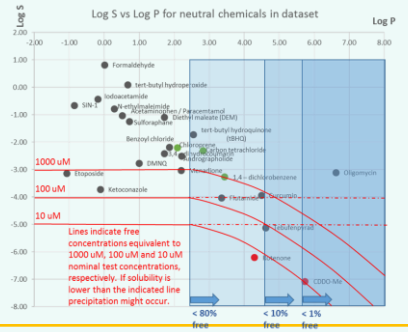
- **The 5th percentile of the <u>BER distribution ranged between 158 and 96738</u>**

Can we develop a general toolbox for estimating BERs?

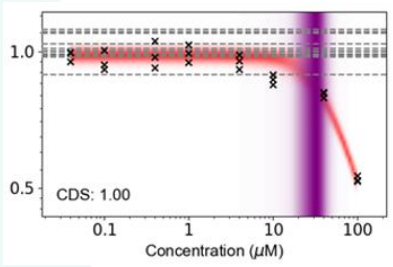
PBK models



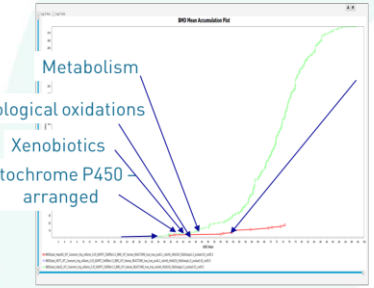
Free concentration



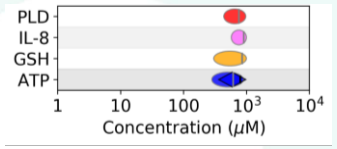
Conc. Resp. models



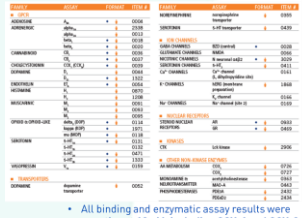
HTTr



CSP

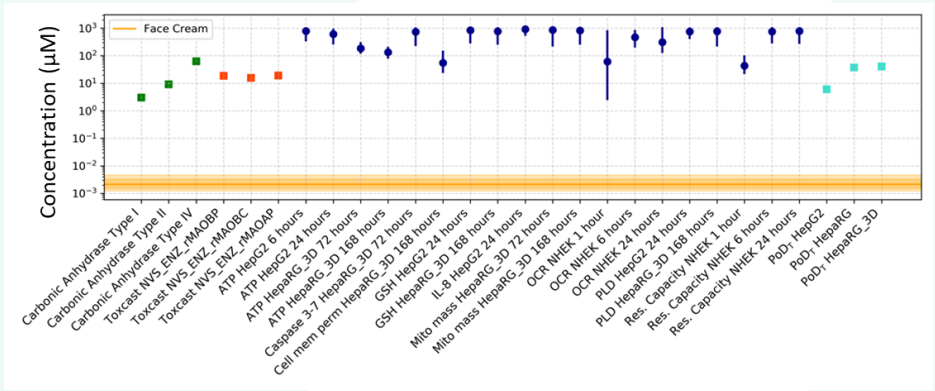


IPP



- 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

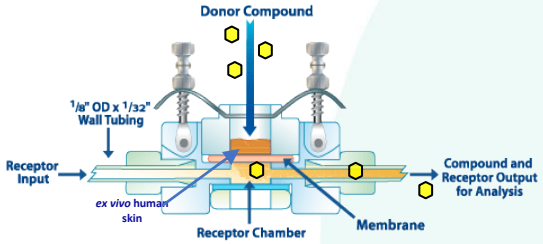


Inform safety decision

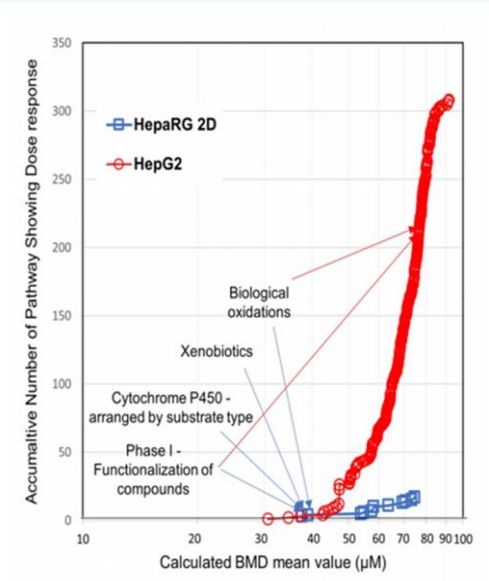
HTTr: High-throughput transcriptomics **CSP:** Cell Stress Panel **IPP:** In vitro pharmacological profiling

Next Generation Risk Assessment is highly interdisciplinary

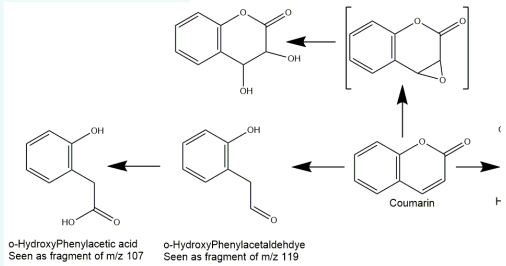
Risk assessment



Biology



Bioinformatics



Chemistry

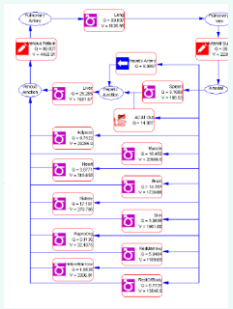
$$y_t = \underbrace{\begin{bmatrix} w_{g,1}^{(1)} & \cdots & w_{g,1}^{(m)} \\ \vdots & & \vdots \\ w_{g,n_y}^{(1)} & \cdots & w_{g,n_y}^{(m)} \end{bmatrix}}_{C} \underbrace{\begin{bmatrix} \phi_g^{(1)}(x_t, u_t) \\ \vdots \\ \phi_g^{(m)}(x_t, u_t) \end{bmatrix}}_{\bar{\varphi}_g(x_t, u_t)} + e_t.$$

Mathematical and statistical modelling

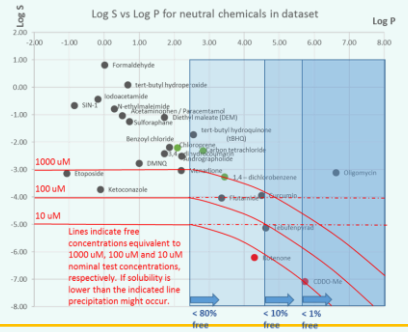
Unilever

Back to the toolbox

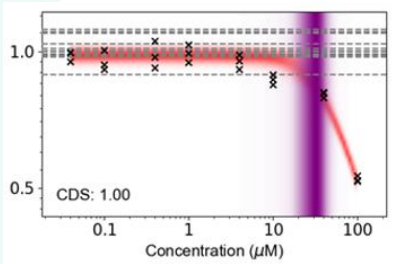
PBK models



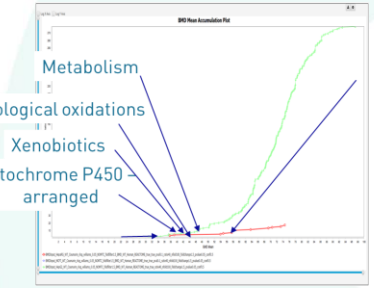
Free concentration



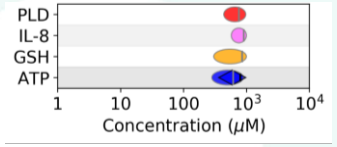
Conc. Resp. models



HTTr



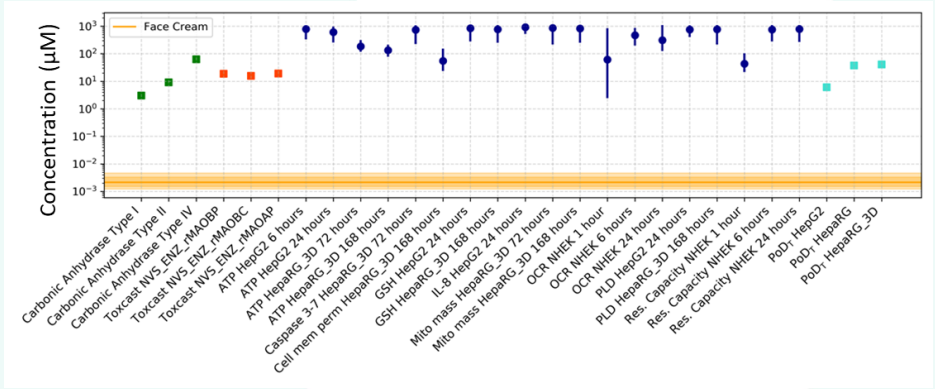
CSP



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

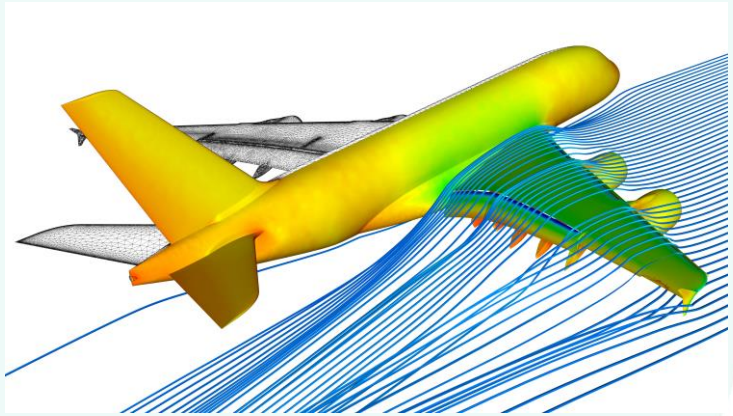


Inform safety decision

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

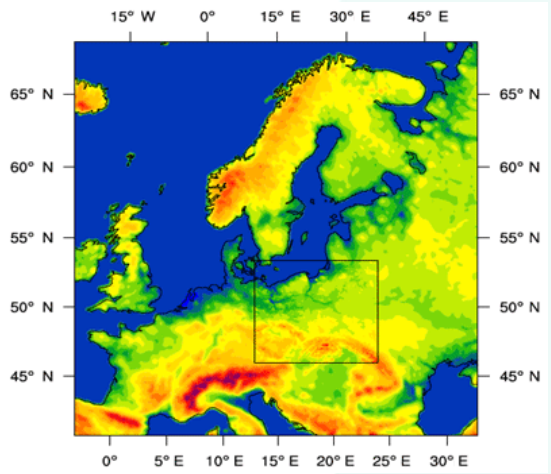
Computational models and their impact on everyday life

Air transport



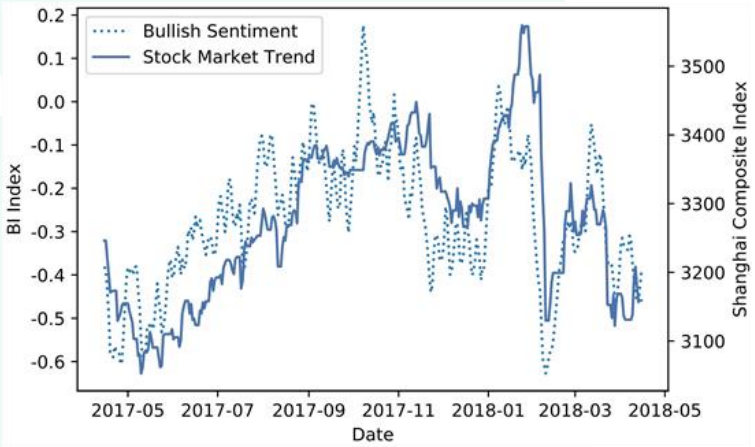
dlr.de

Weather forecast

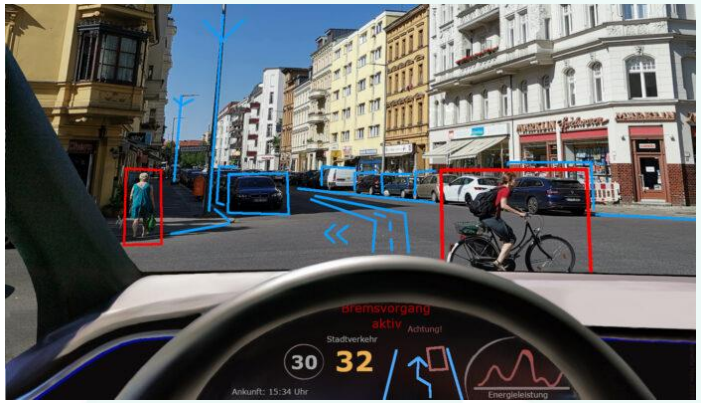


Satnav

Stock market



Self driving cars



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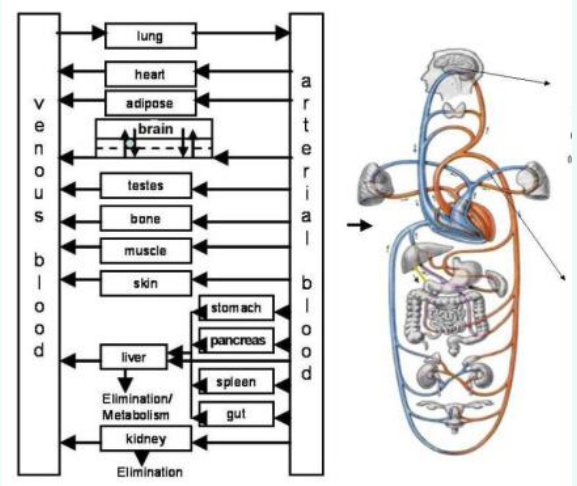
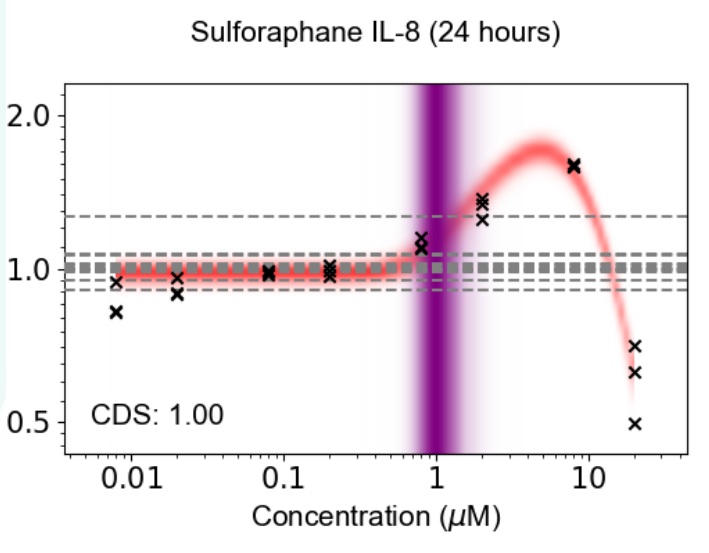
Different types of computational approaches used in NGRA

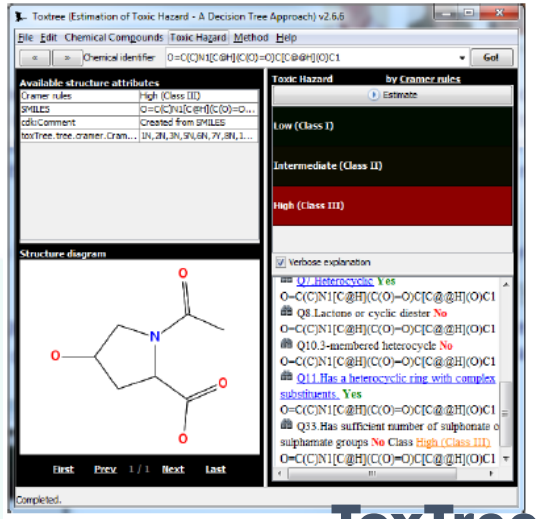
Physiologically-based kinetic (PBK) modelling



Dose response modelling

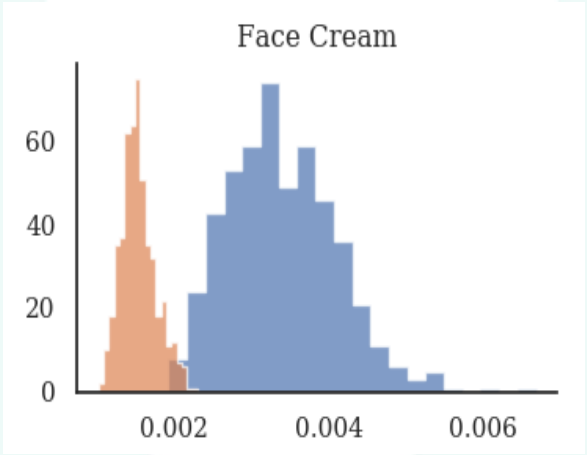


In silico tools

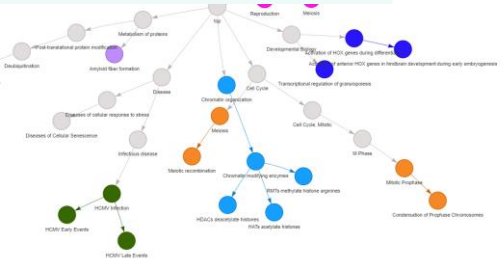


ToxTree

Statistical models of uncertainty and variability



Bioinformatics tools for analysing omics data



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Dose response models

The cell stress panel

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

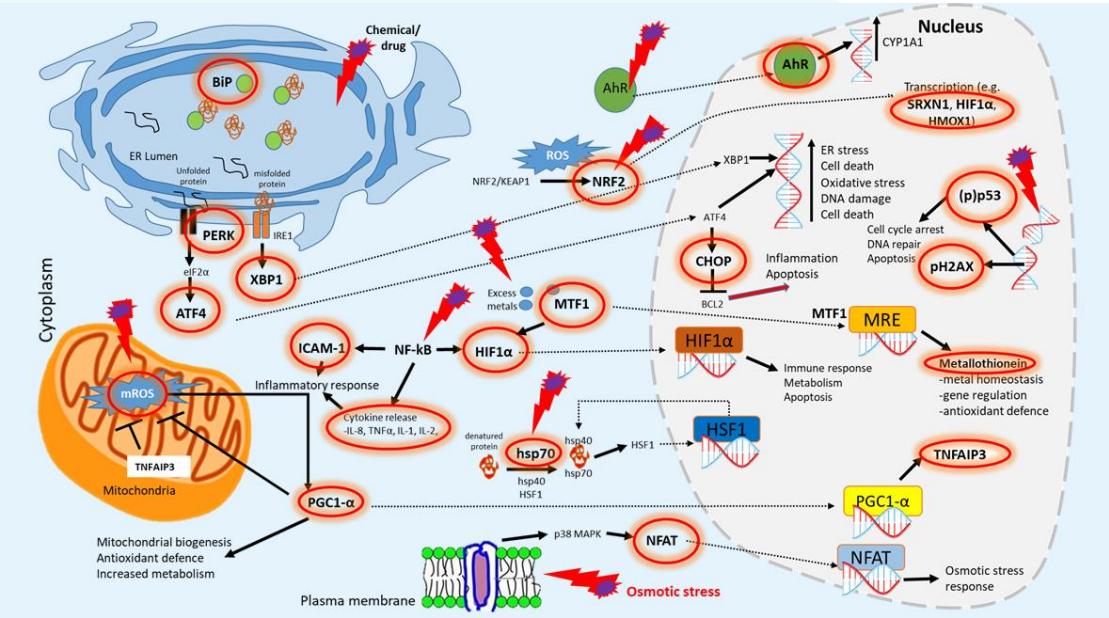

Image kindly provided by Paul Walker (Cyprotex)

OXFORD SOT Society of Toxicology
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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

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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.

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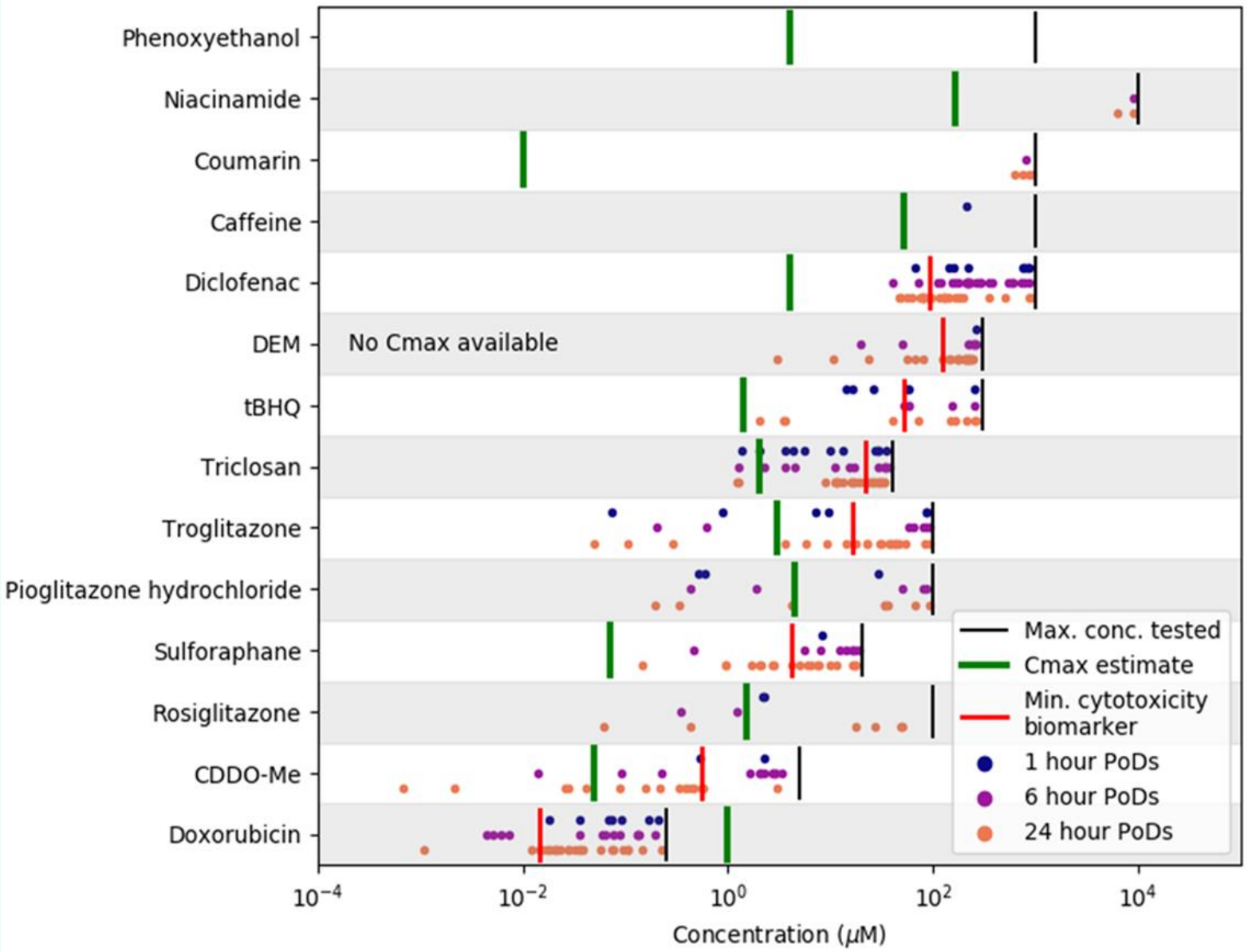
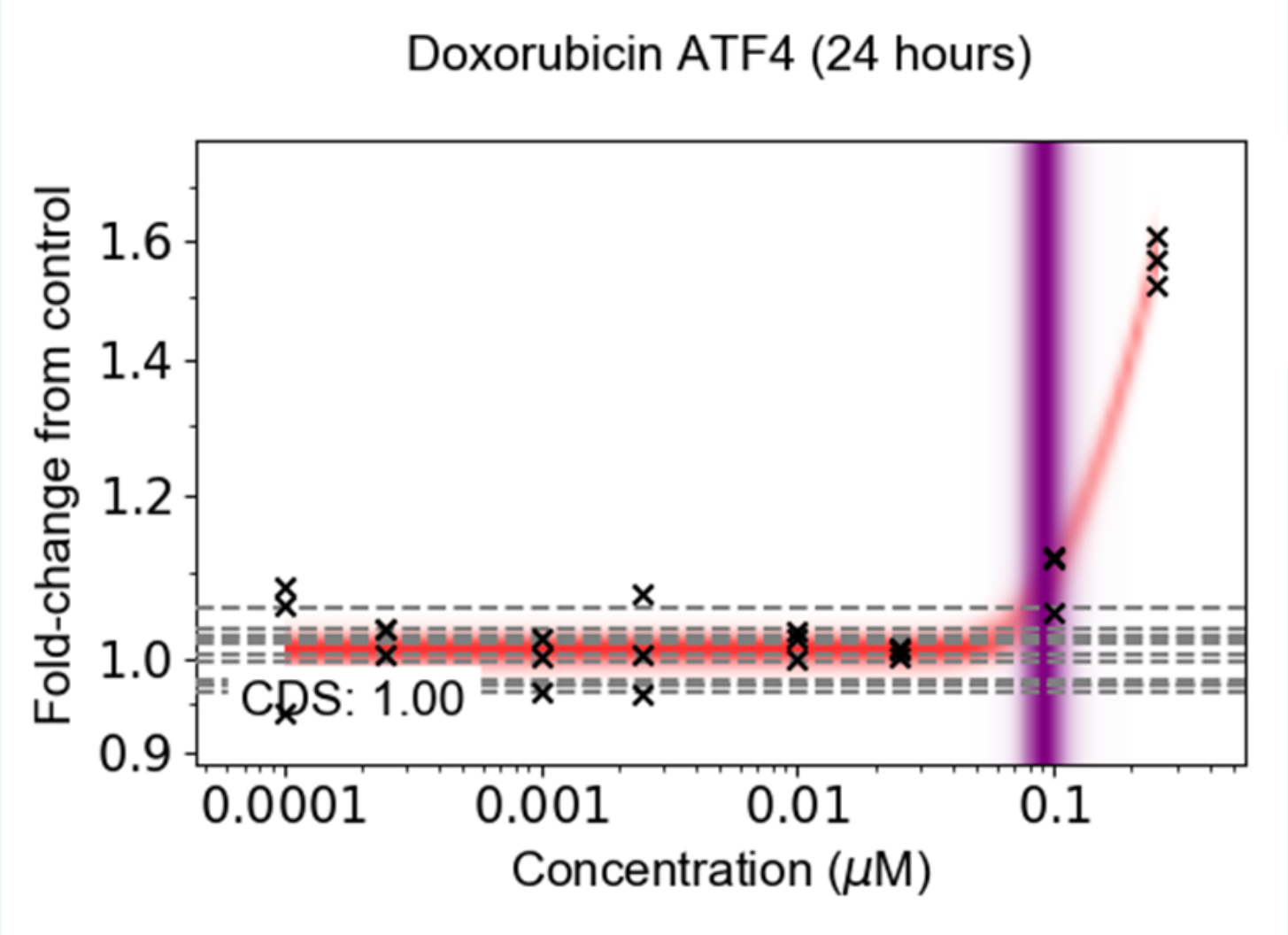


Hatherell *et al.*, 2020, Identifying and characterizing stress pathways of concern for consumer safety in next generation risk assessment, Tox. Sci. https://doi.org/10.1093/toxsci/kfaa054

Dose response analysis and estimating PODs



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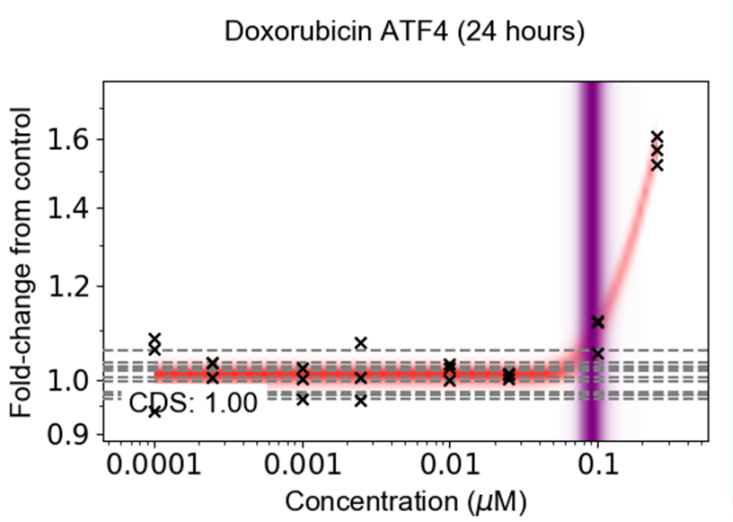
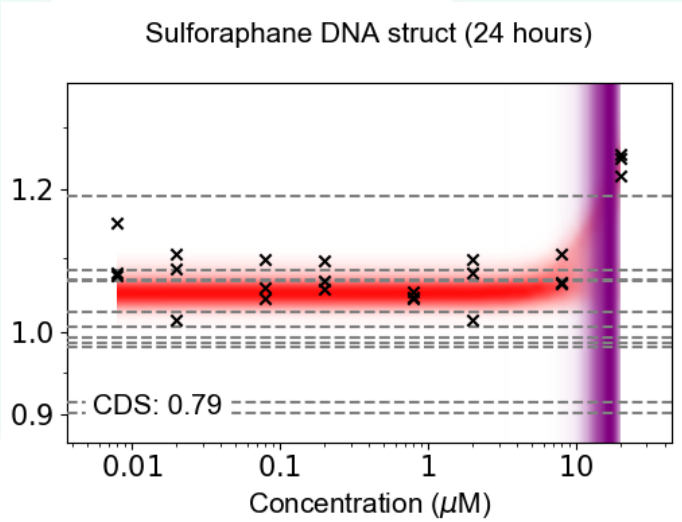
Dose response analysis and estimating PODs

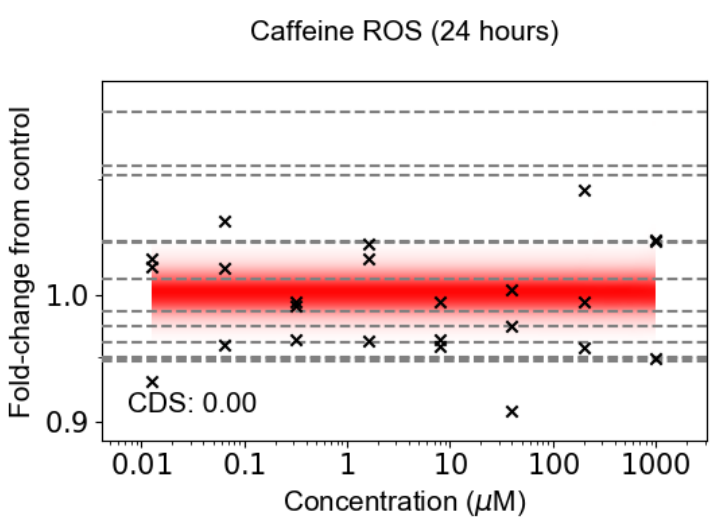
Clear effect



Is there an effect here?



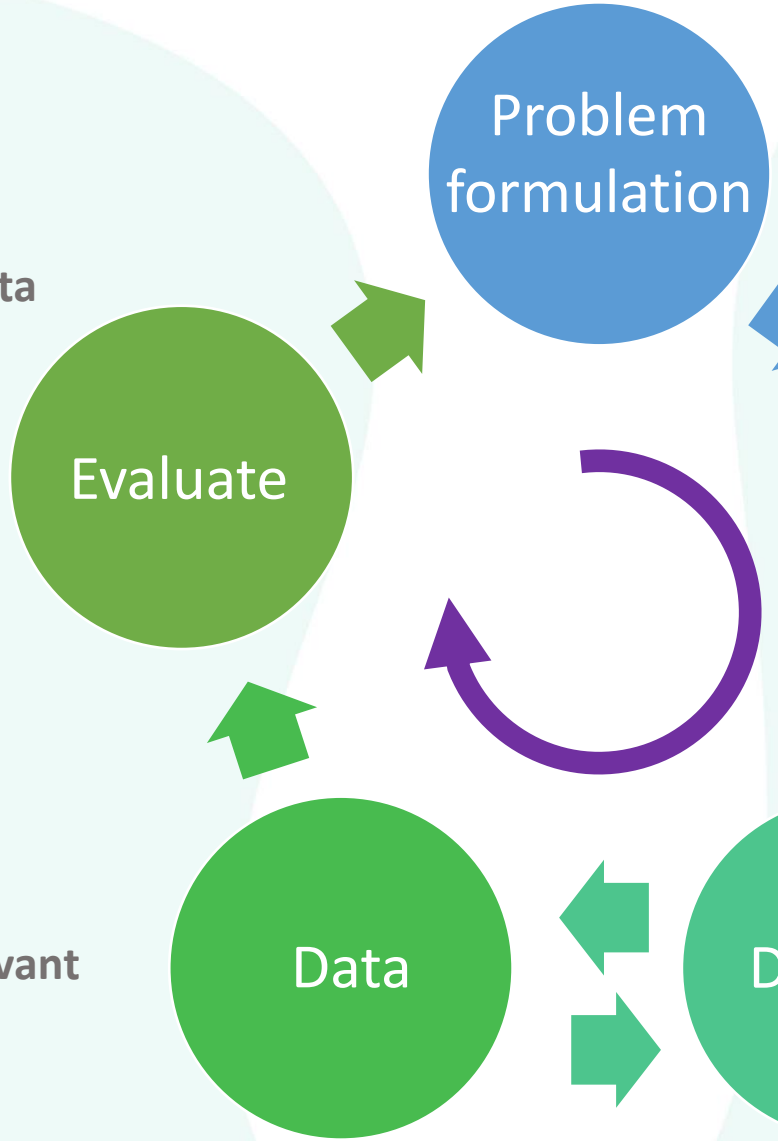
No effect



- 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

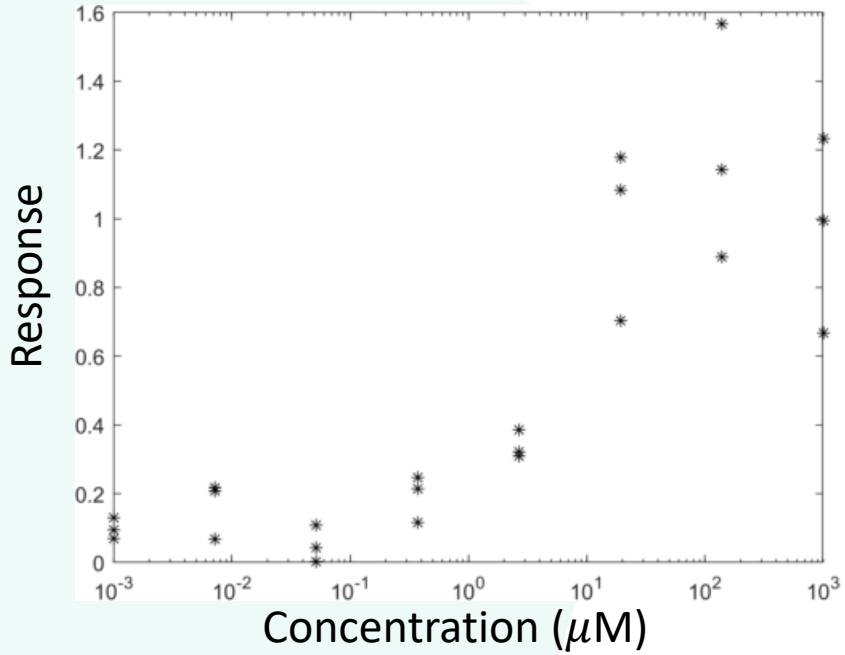
Develop and implement the model

Generate/curate relevant data

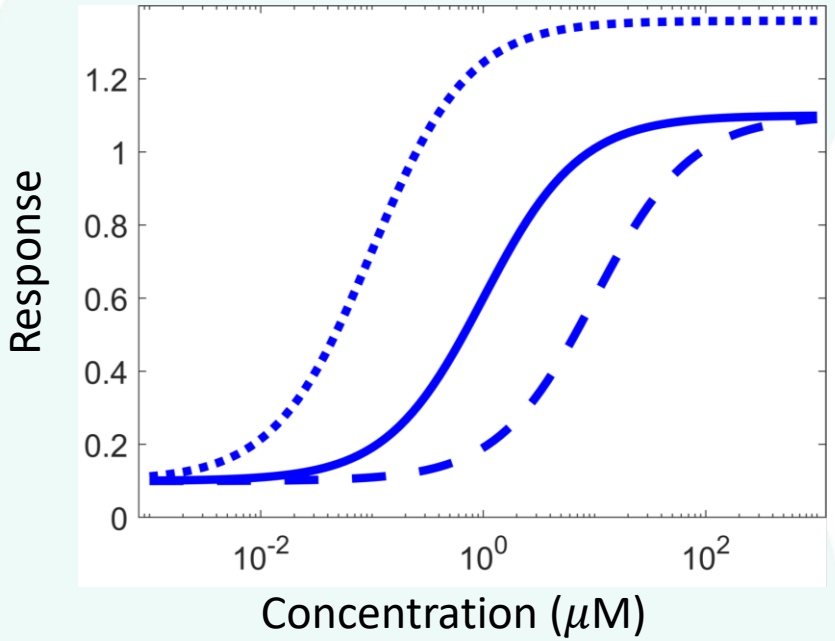
How does the model perform?
Does it describe the data well?

Developing a dose response model

Example dose response data



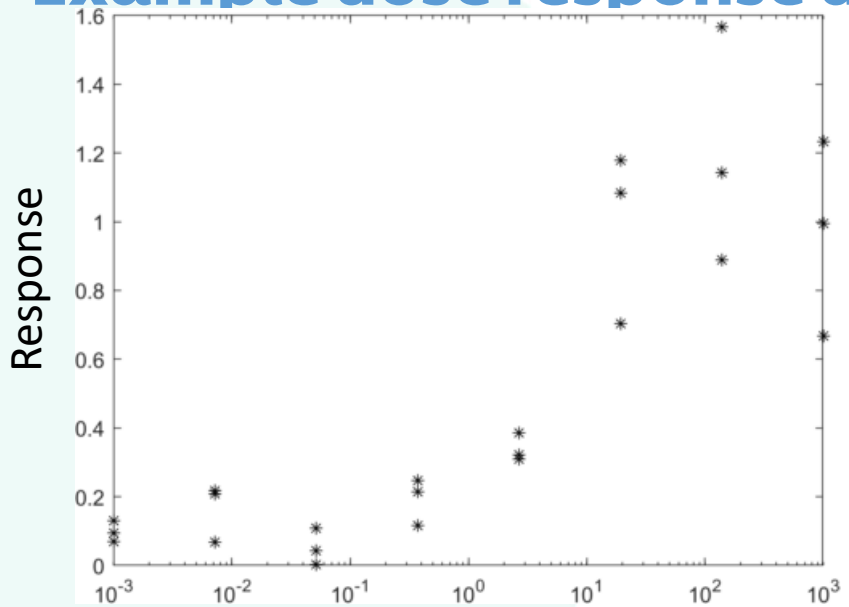
Hill function



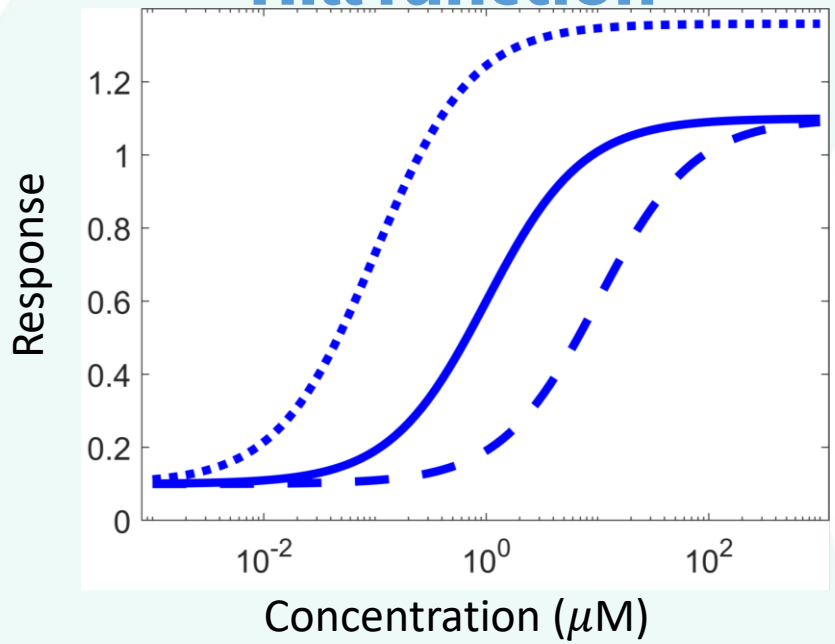
- **Problem**: We want to know:
  - Does the chemical have an effect on our biomarker
  - At what concentration does this occur?
  - We want to quantify the uncertainty in these.
- **Assumption**: There is an increase in our biomarker, which can be captured using a Hill function.

Back to the dose response example

Example dose response data



Hill function

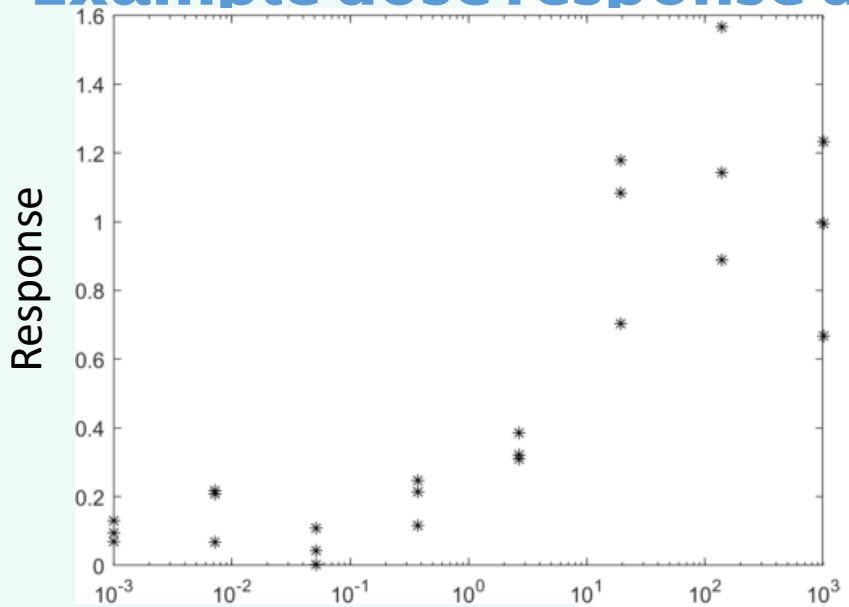


Develop

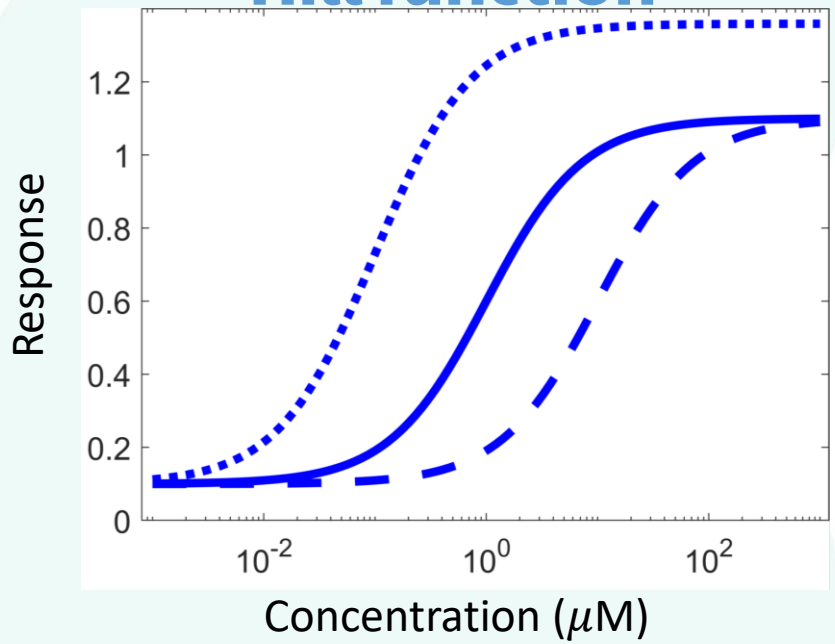
- Main building blocks of the model:
  - Measured data = Mean Response + Observational Noise
  - $y = f(x|C, \theta, V_{max}) + \eta$
- y and x are the observations and concentrations respectively.
- Assume η is normally distributed with standard deviation σ

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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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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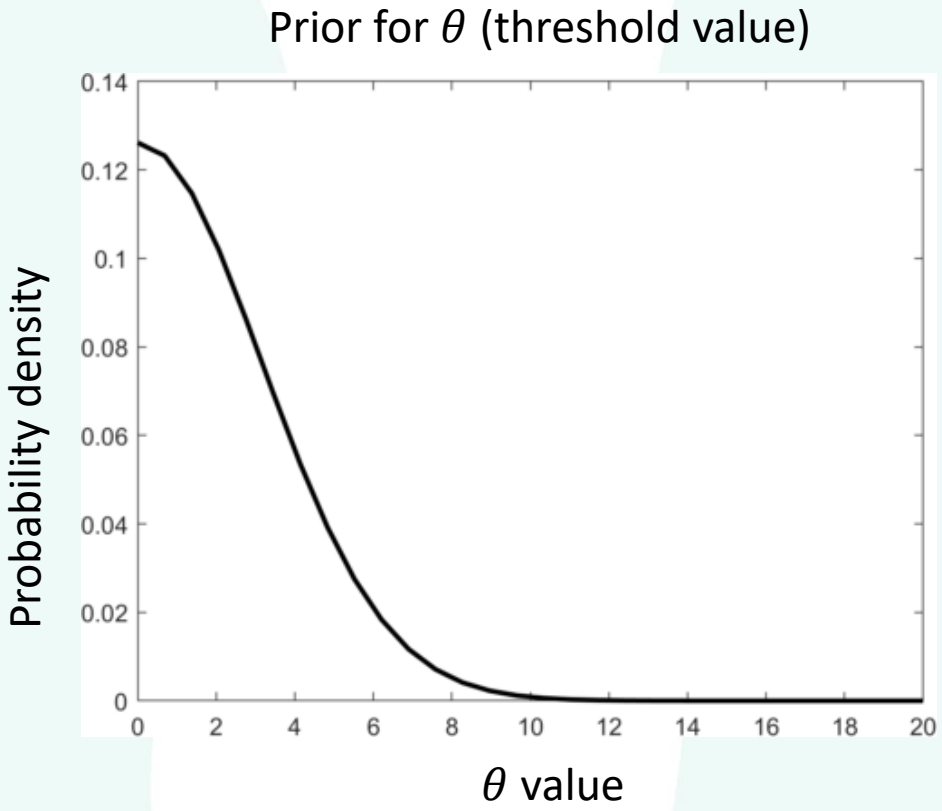
Example of a prior

Develop

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

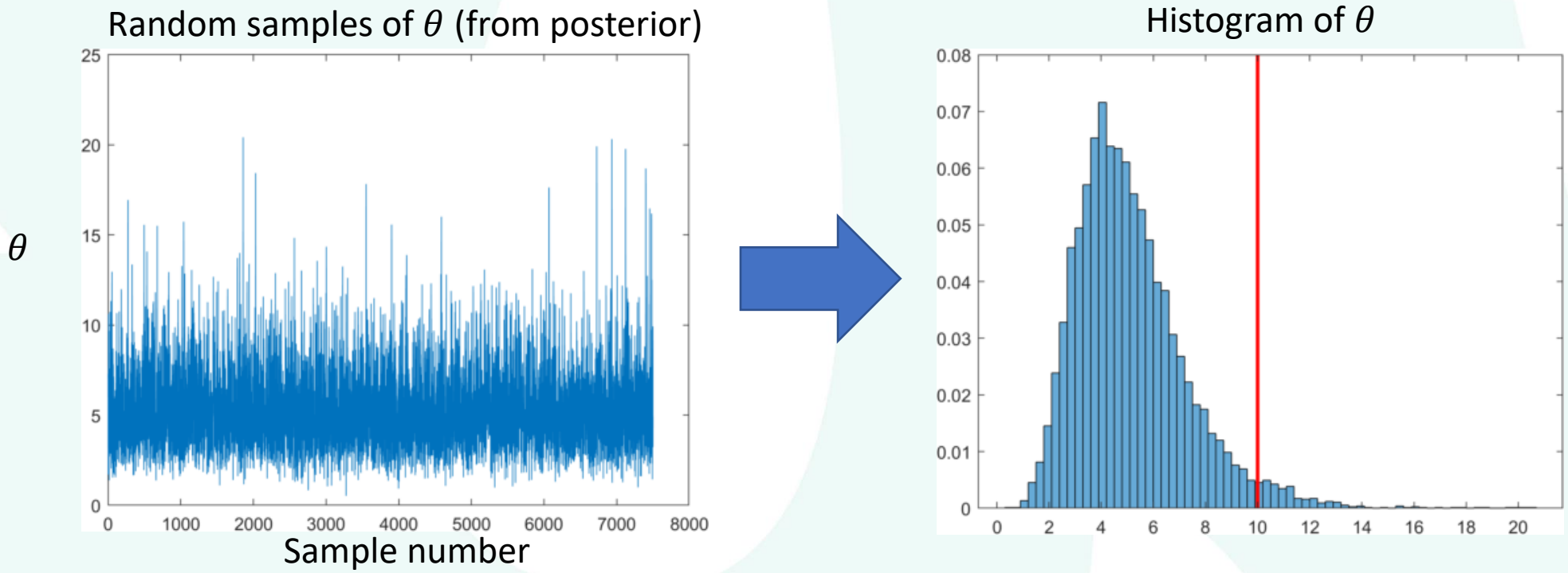


Data

- 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.

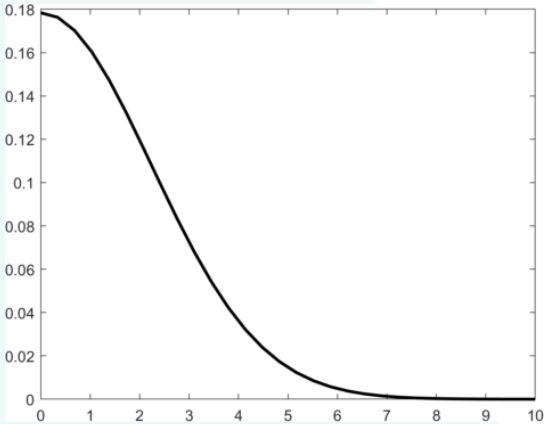


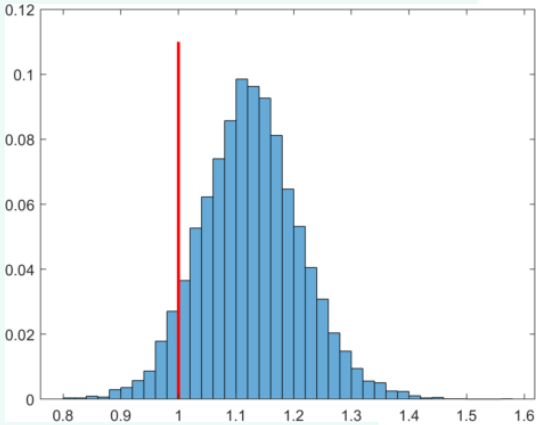
Learning parameters from the data: prior vs posterior

Prior

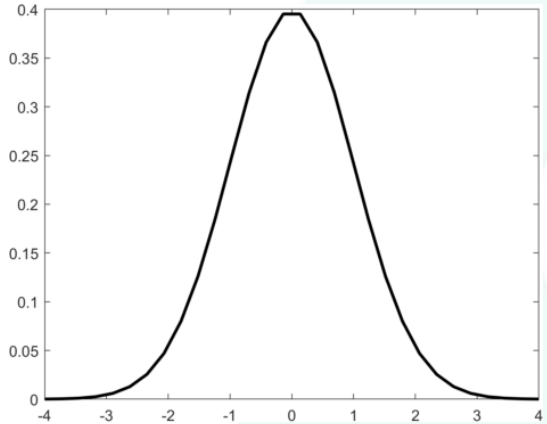
Posterior

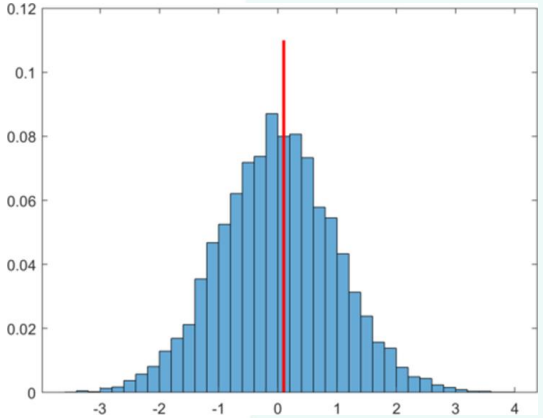
V_{max}



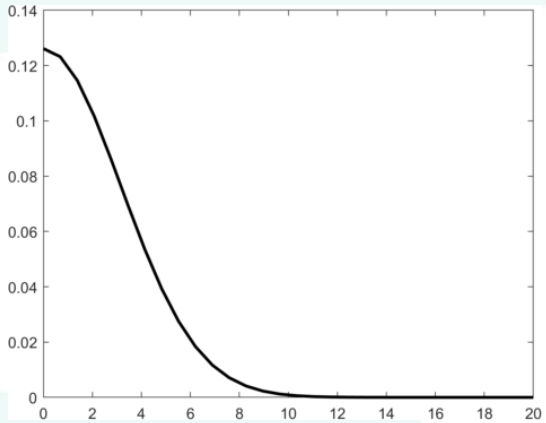


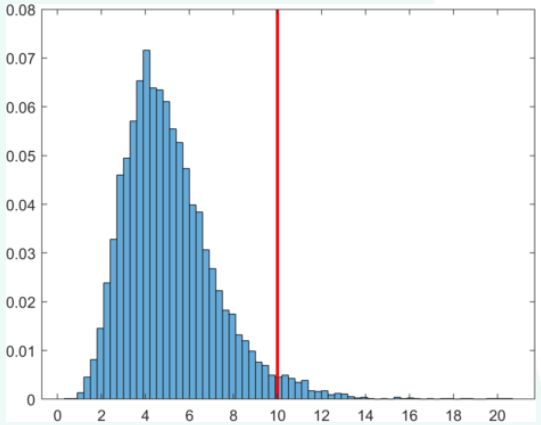
C





θ





Red horizontal line indicates the ‘true’ value

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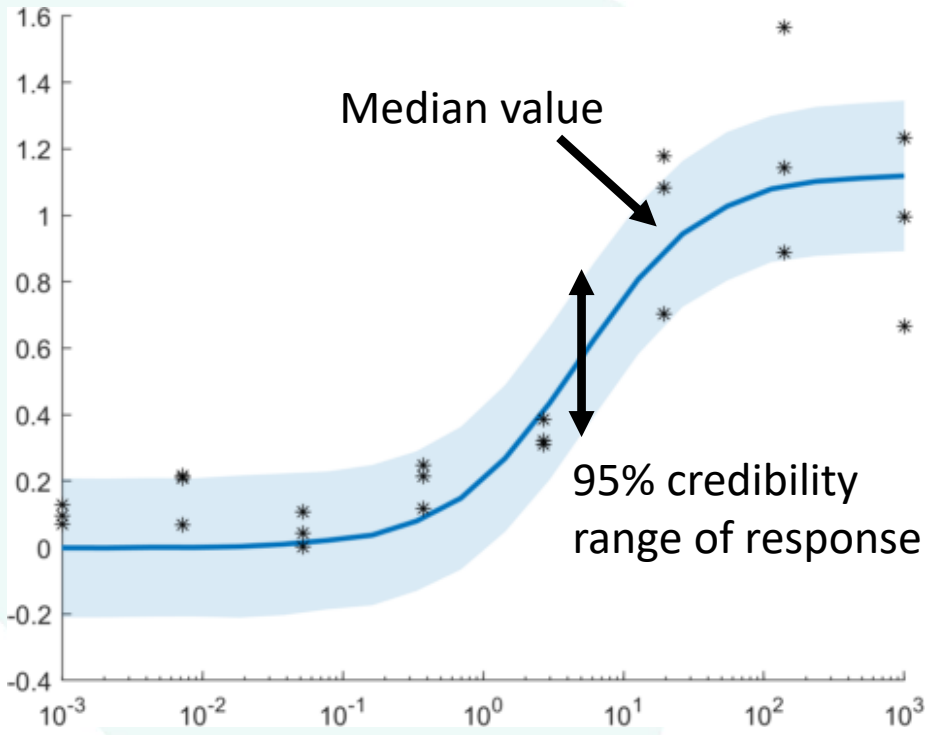
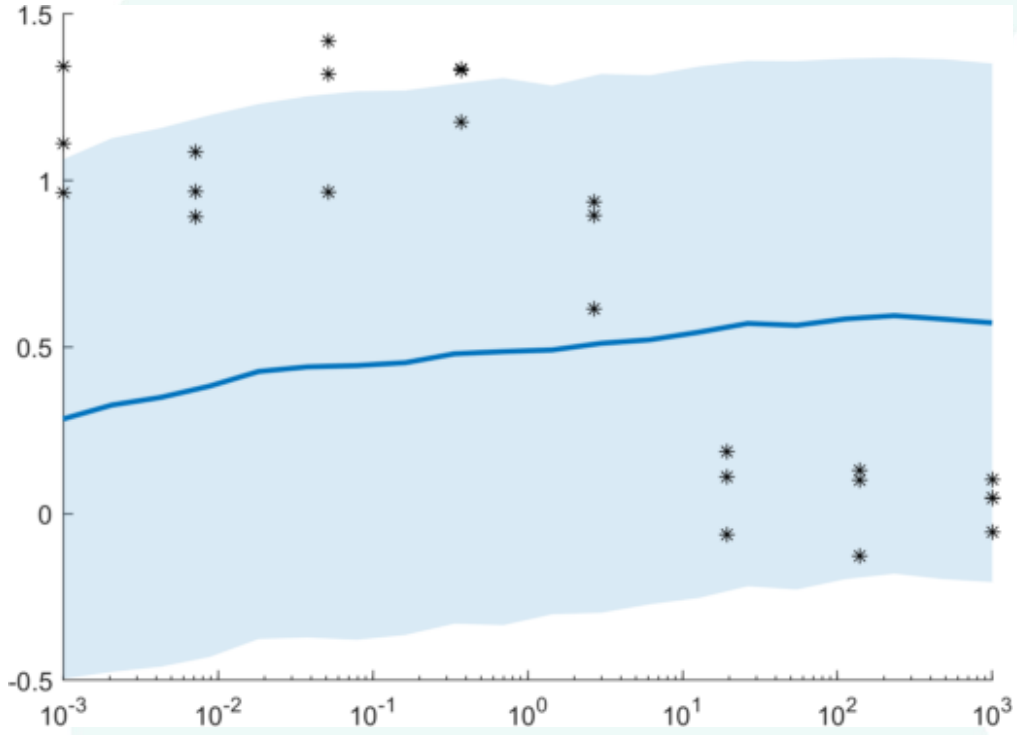
Evaluating the dose response model





- 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

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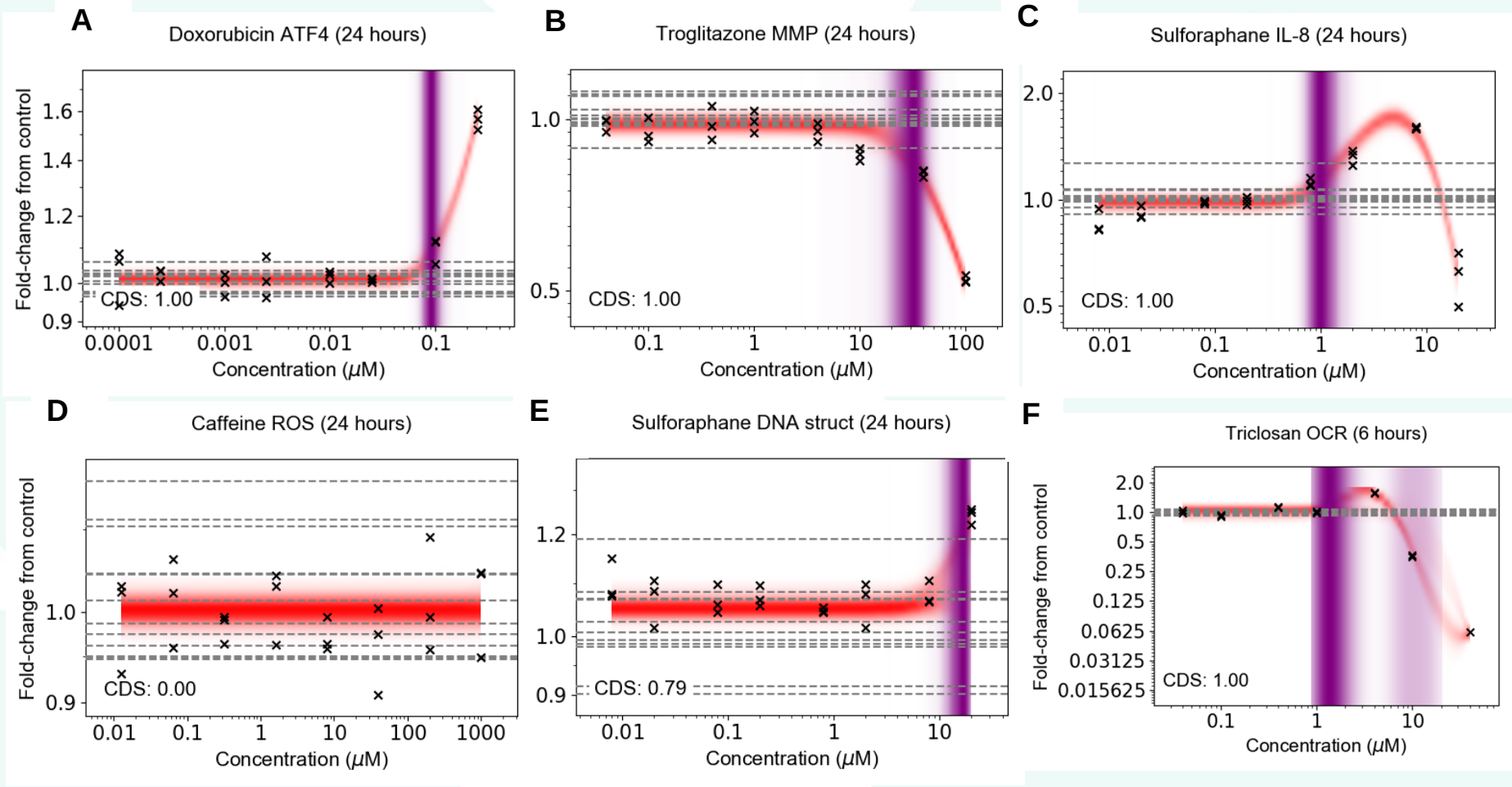
Back to the cell stress panel



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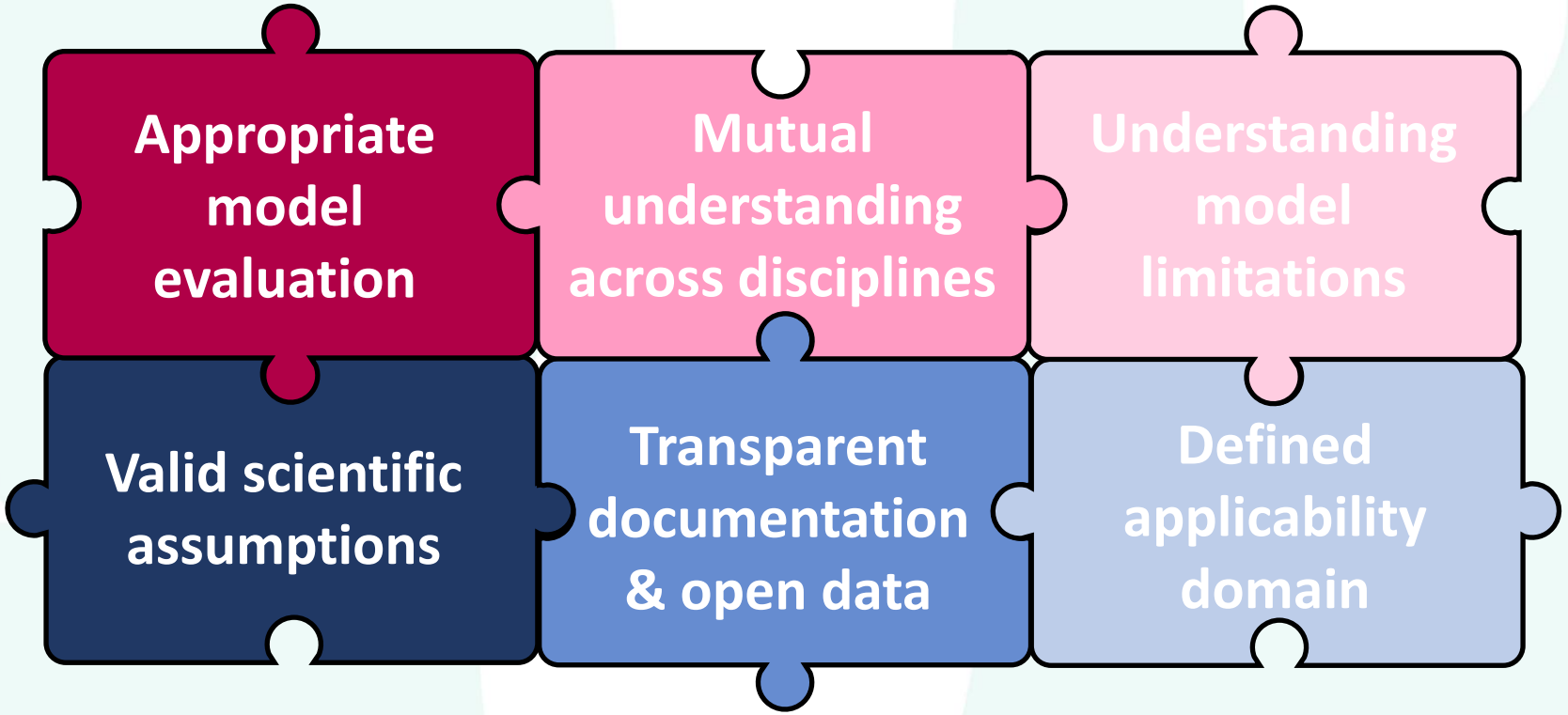
Challenges in the acceptance of using computational approaches in NGRA



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Evaluating a toolbox of NAMs

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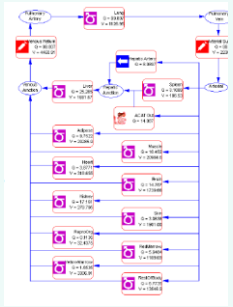
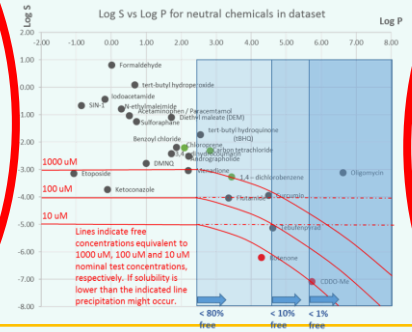
Back to the toolbox

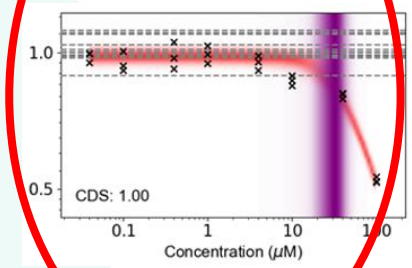
PBK models



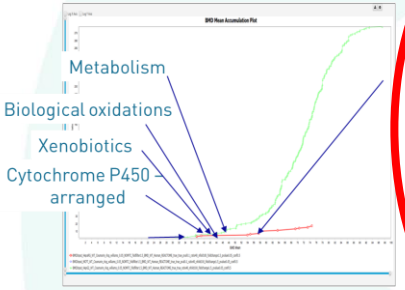
Free concentration



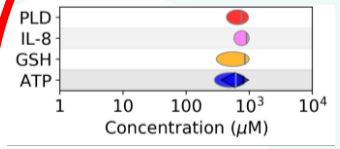
Conc. Resp. models



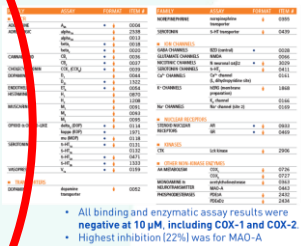
HTTr



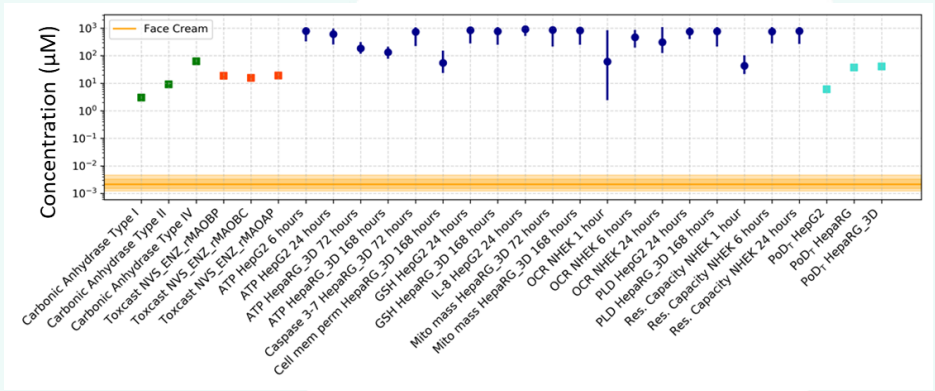
CSP



IPP



Bioactivity exposure ratio



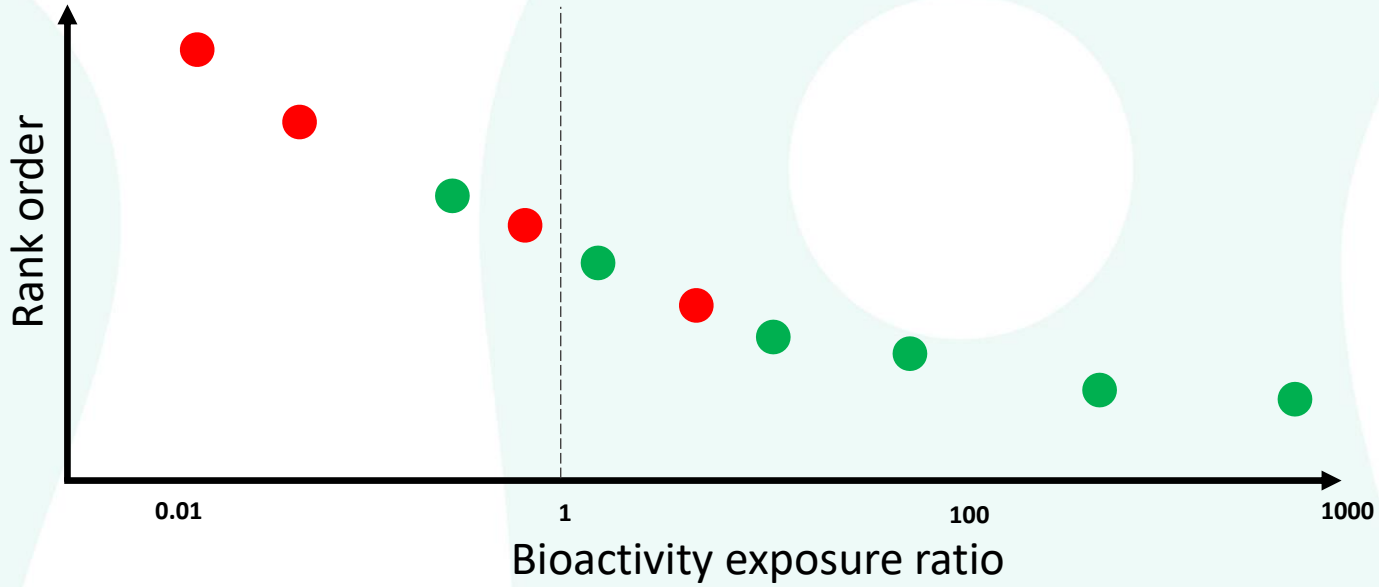
Inform safety decision

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

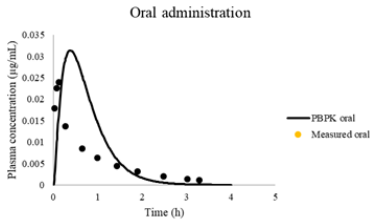
An evaluation strategy for the toolbox

Chemical exposures scenarios

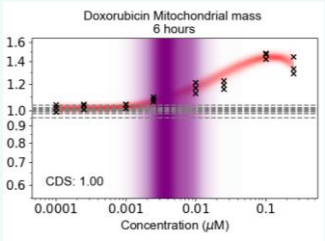
- ‘Low’ risk (from consumer goods perspective) – e.g. foods, cosmetics
- ‘High’ risk (from consumer goods perspective) – e.g. drugs



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



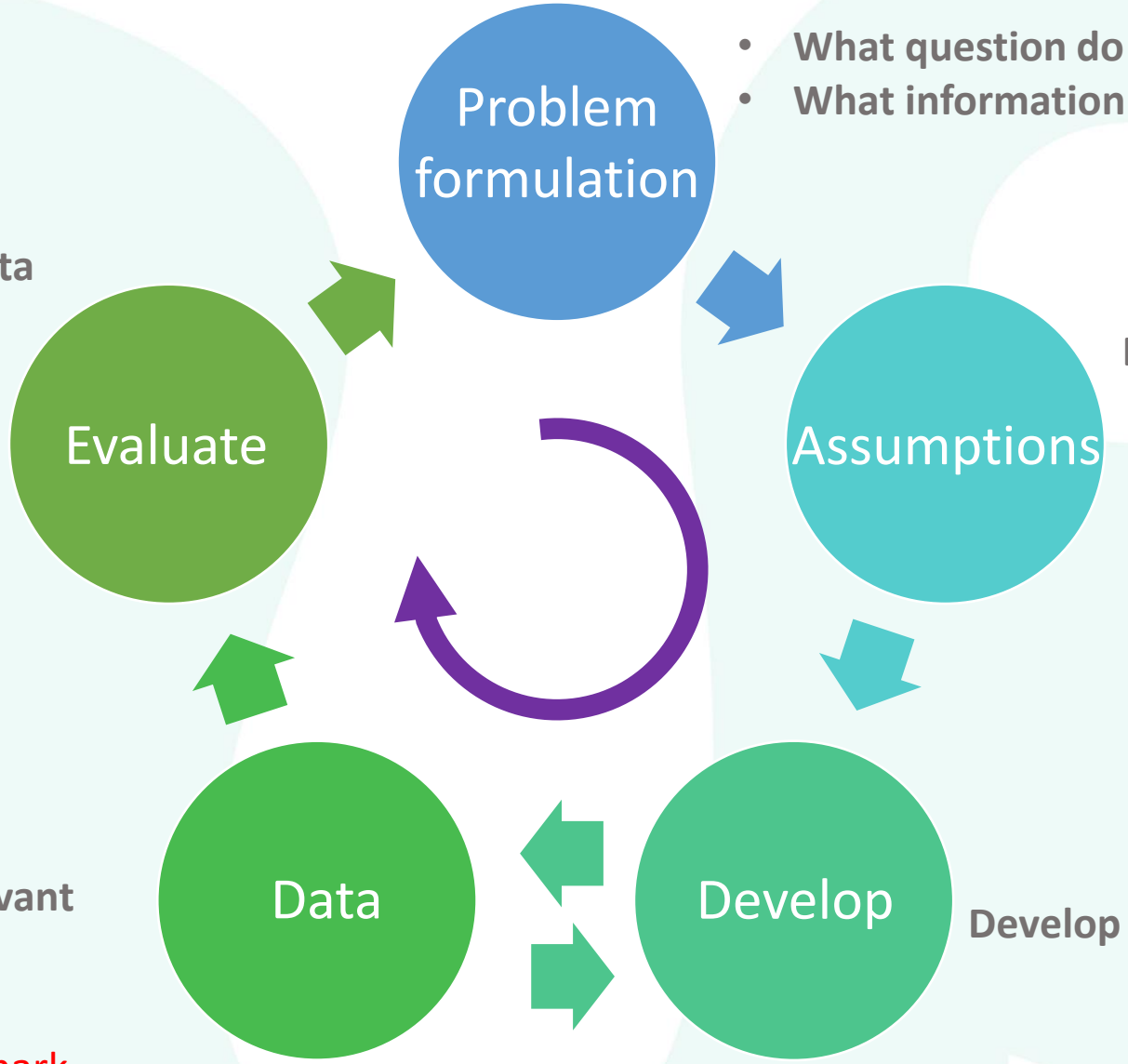
PBK models of systemic exposure



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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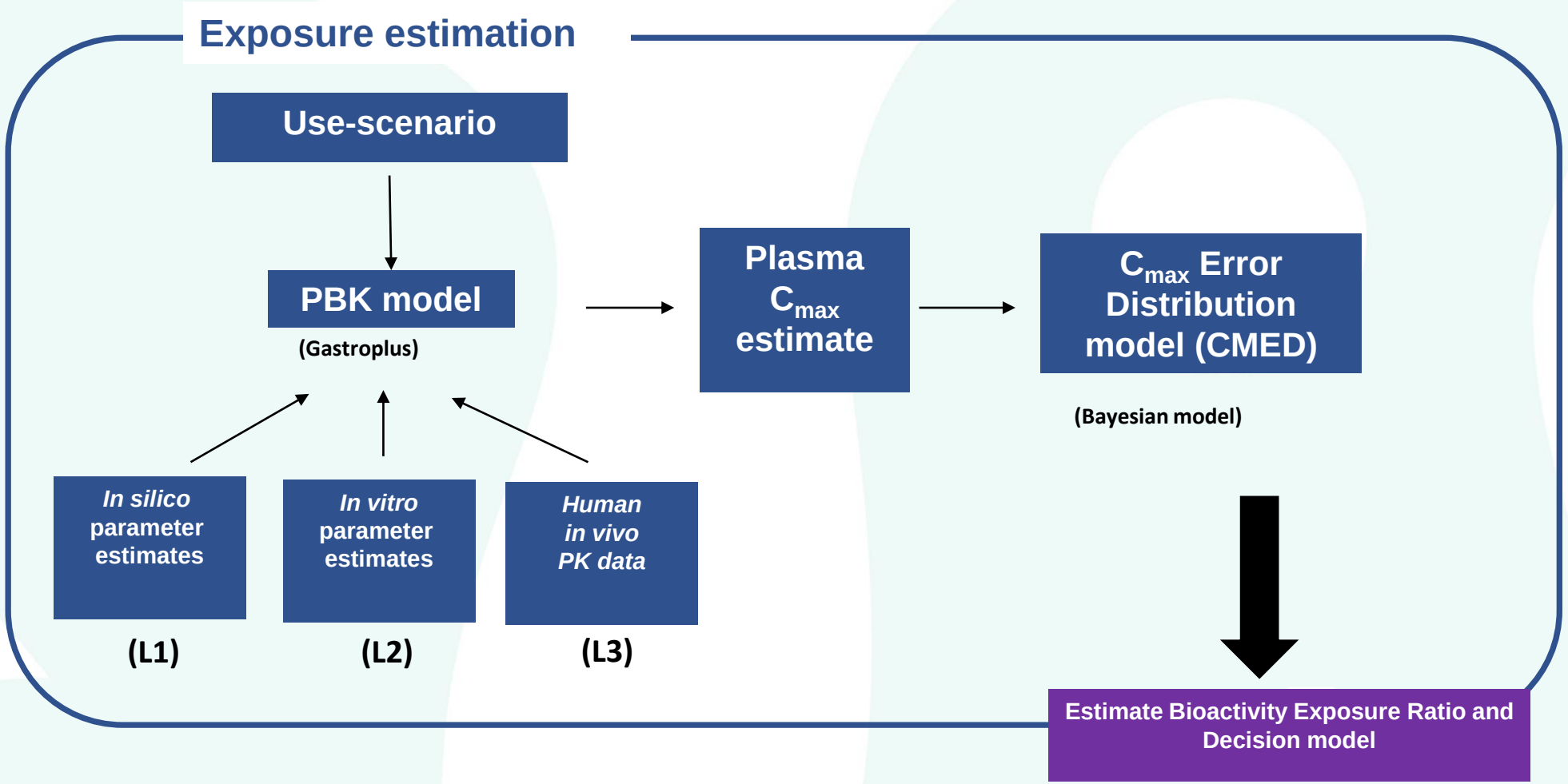
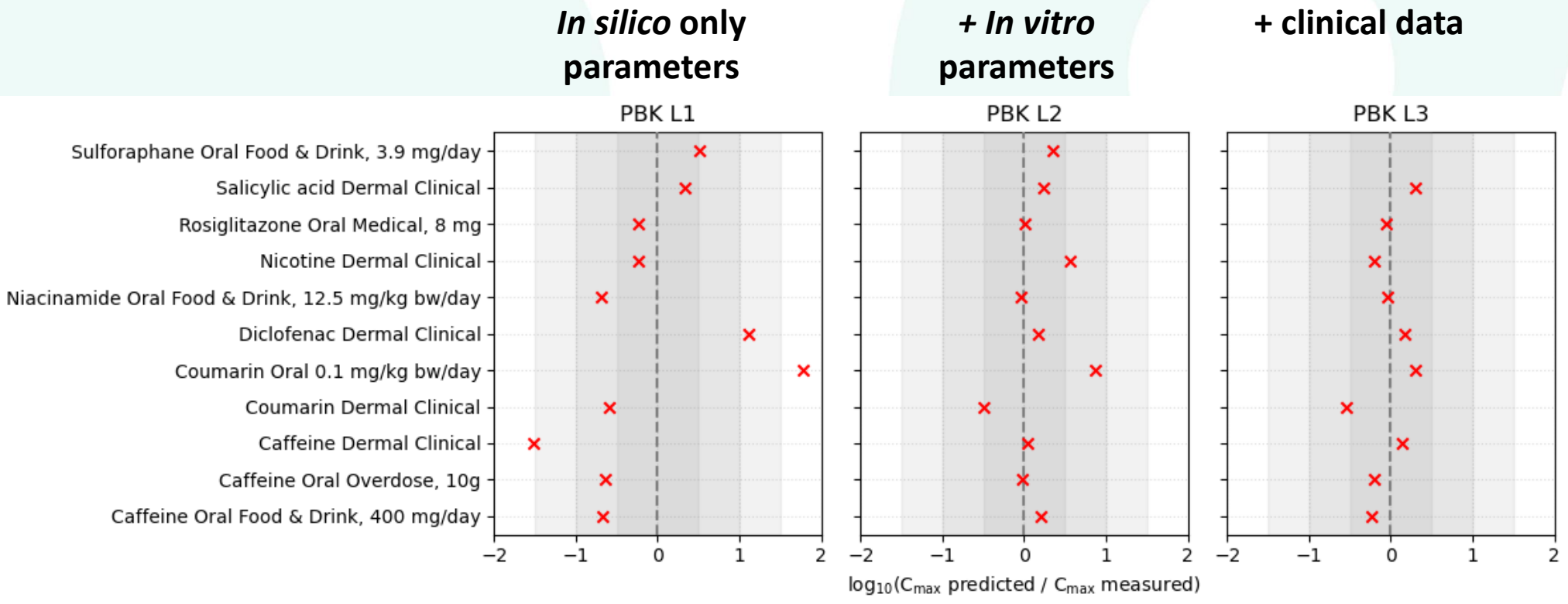
Using PBK models to predict Cmax



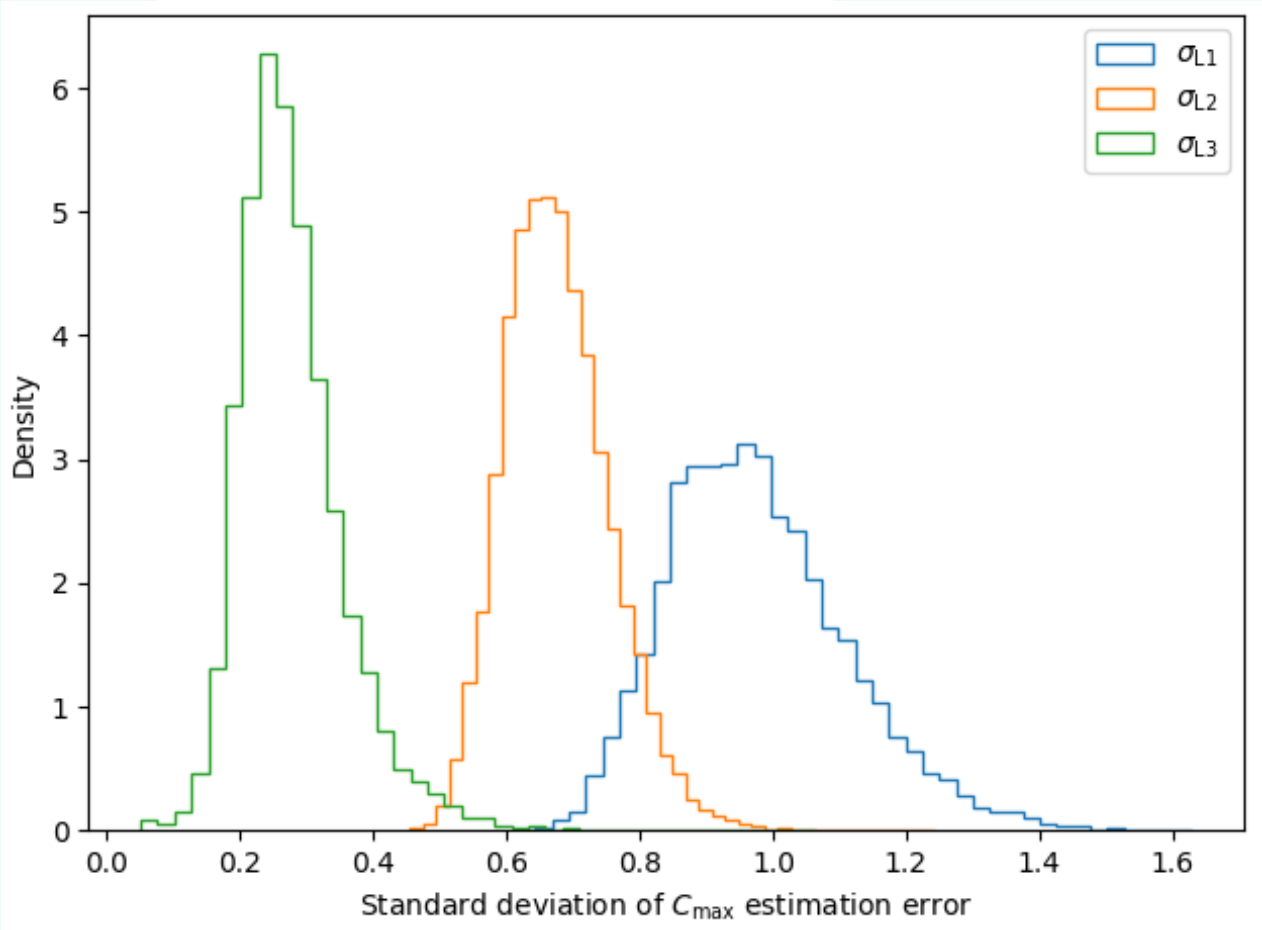
- Used a (**bottom-up**) PBK model to predict Cmax under different parameterisations
- Used a (**top down**) Bayesian statistical model to quantify the potential error in the est

Quantifying the error in the Cmax estimates

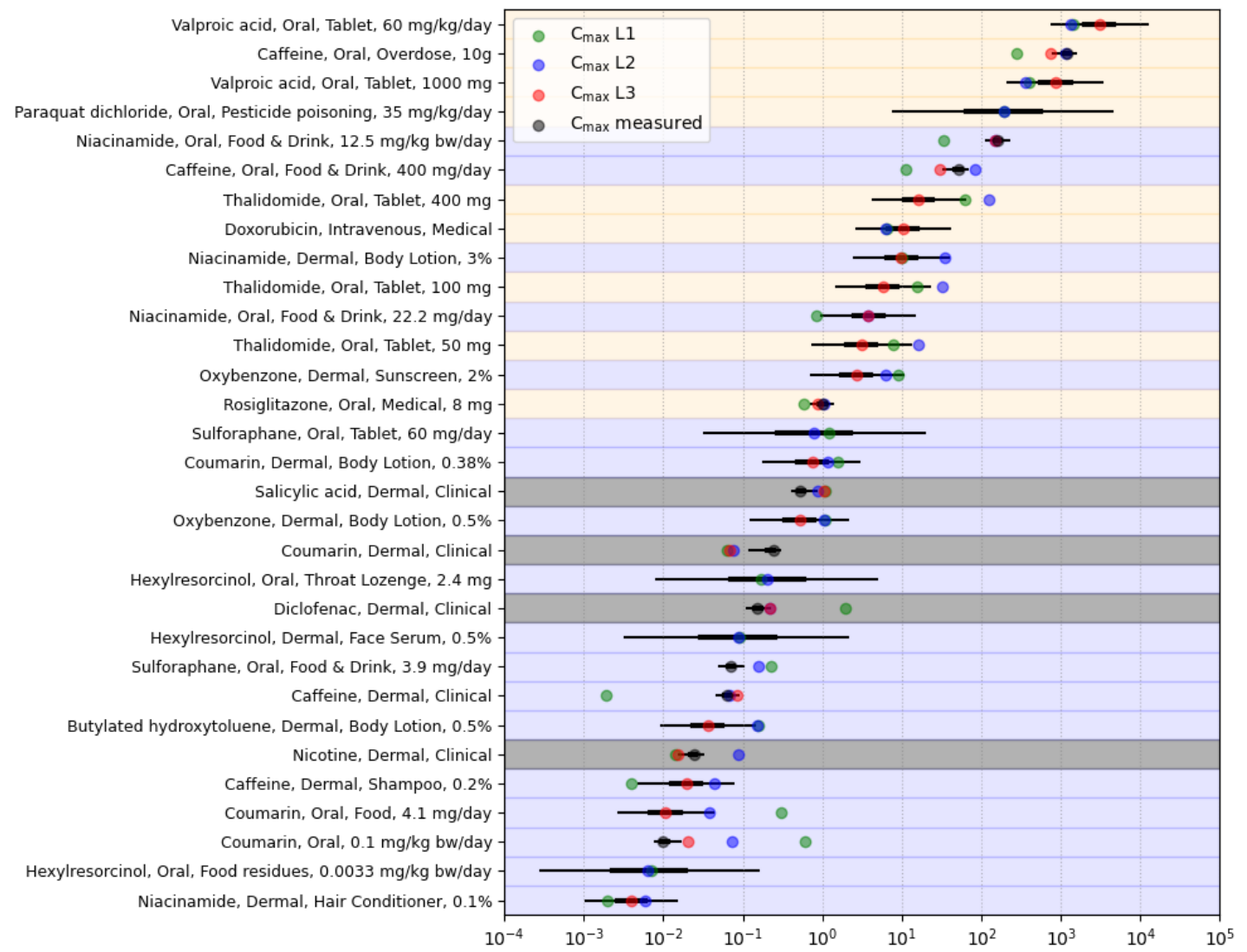


- The PBK prediction error decreases as we go through the different parameterisation levels
- This is an empirical observation

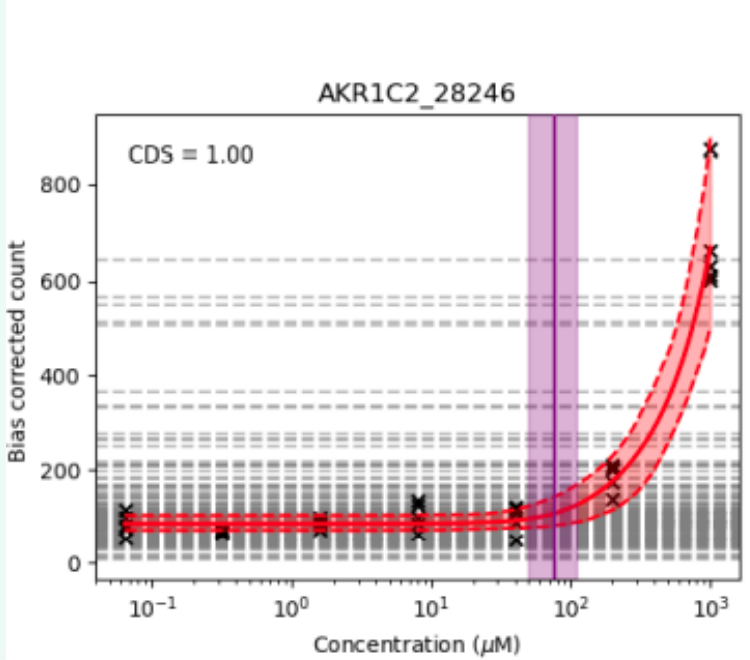
Using a Bayesian model to learn the PBK C_{max} prediction error

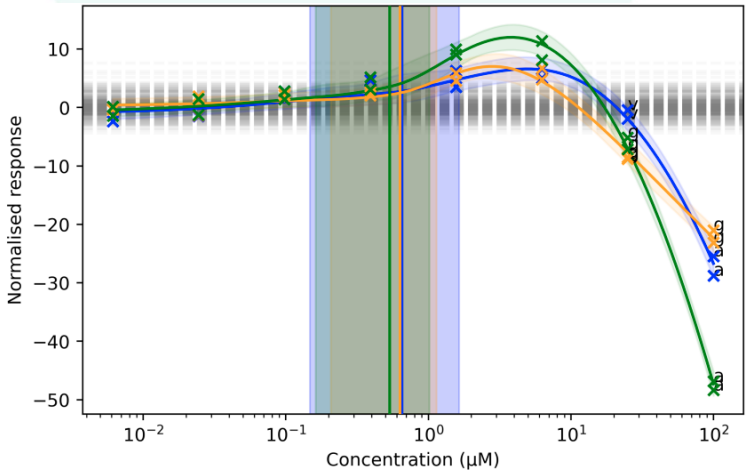


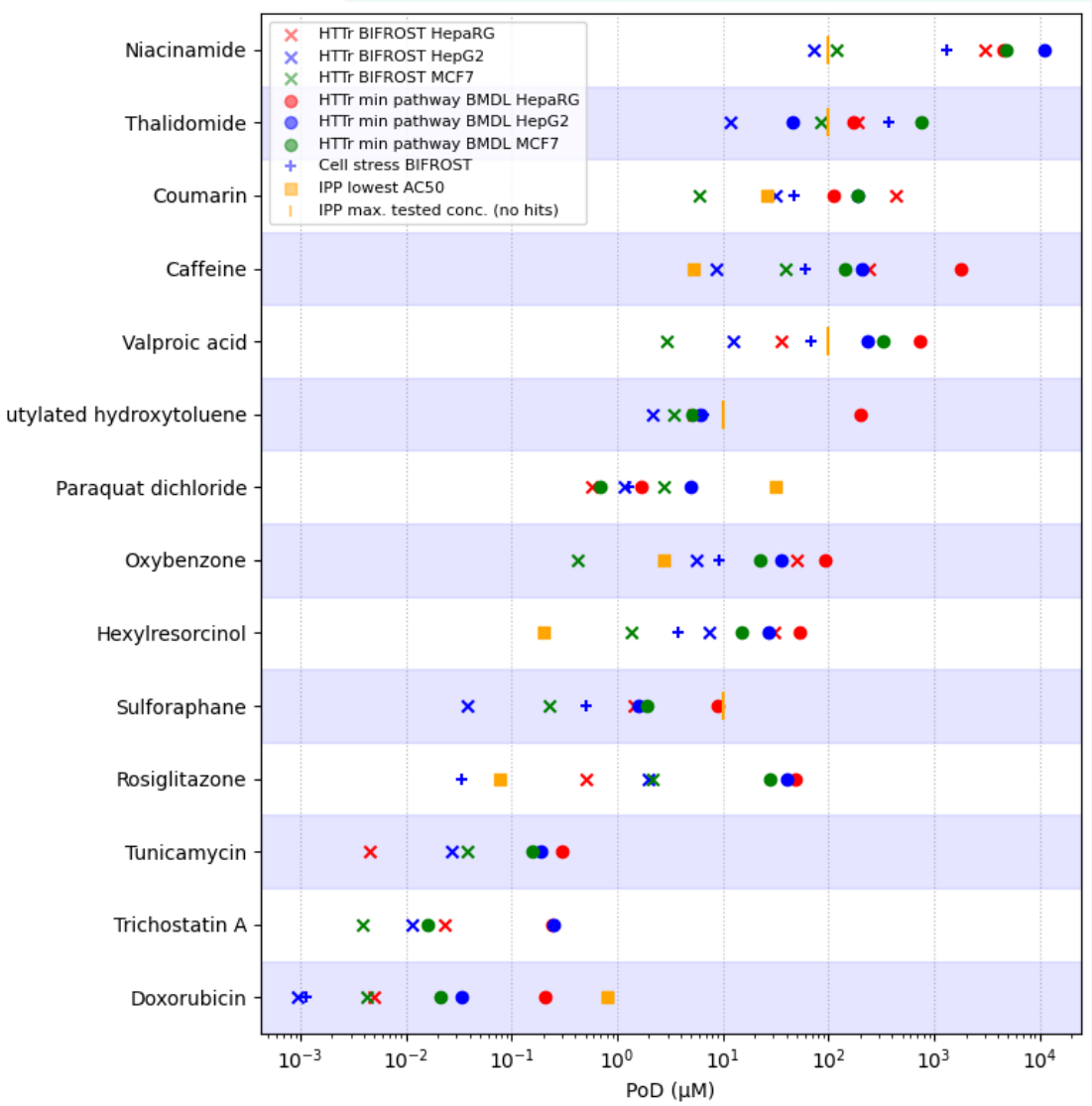
Using PBK models to predict Cmax



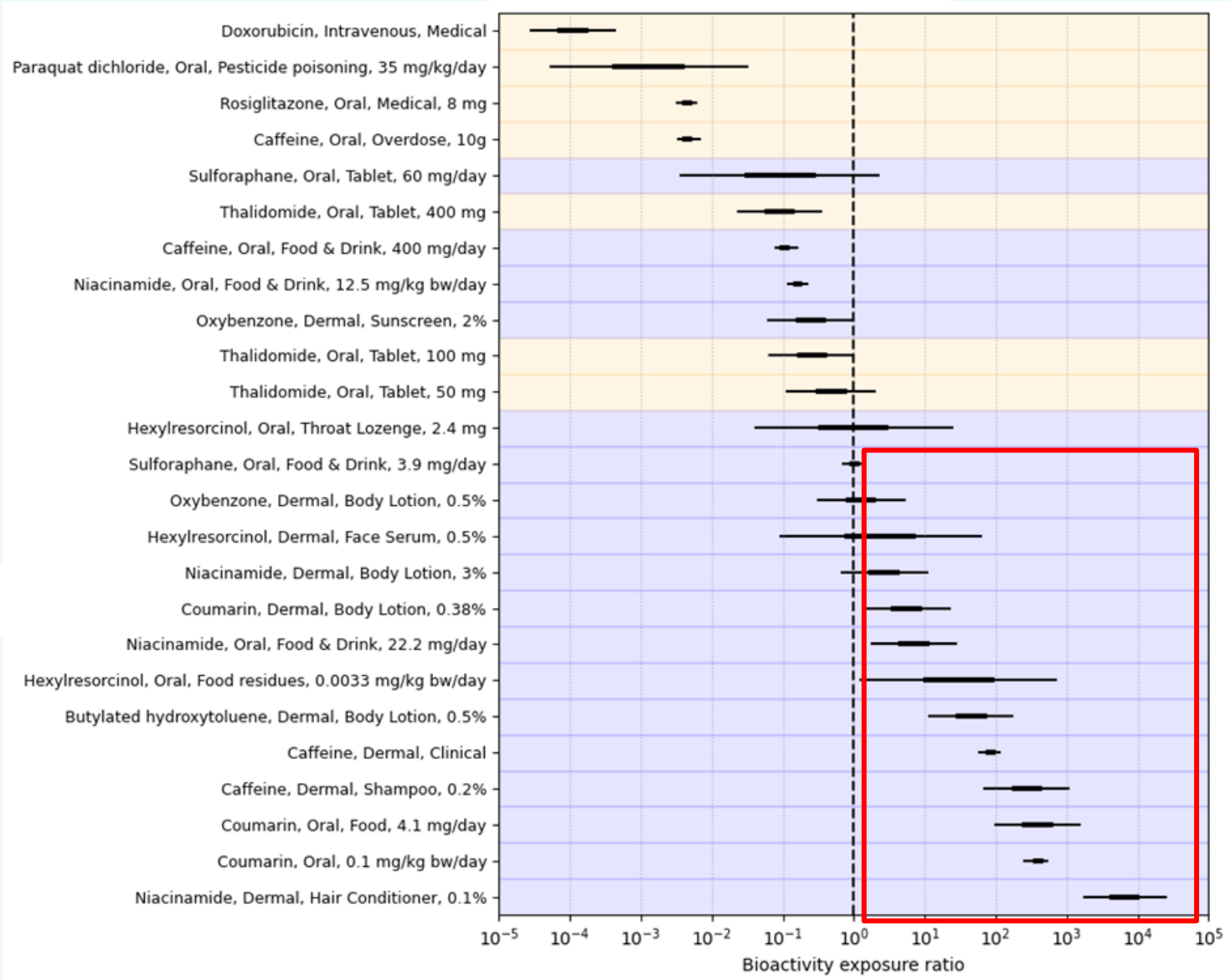
PODS from the bioactivity platforms







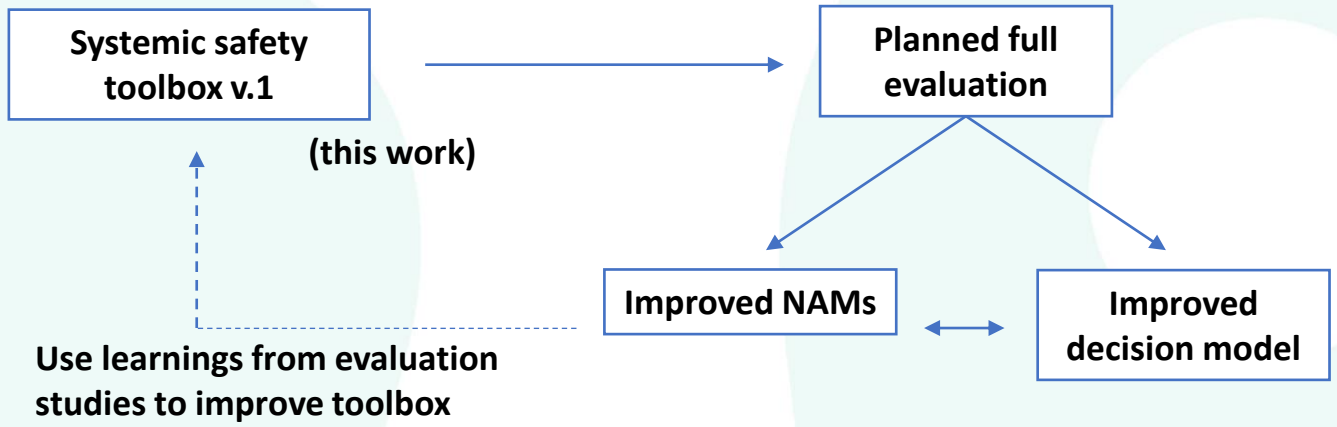
Initial results indicate the toolbox is protective



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

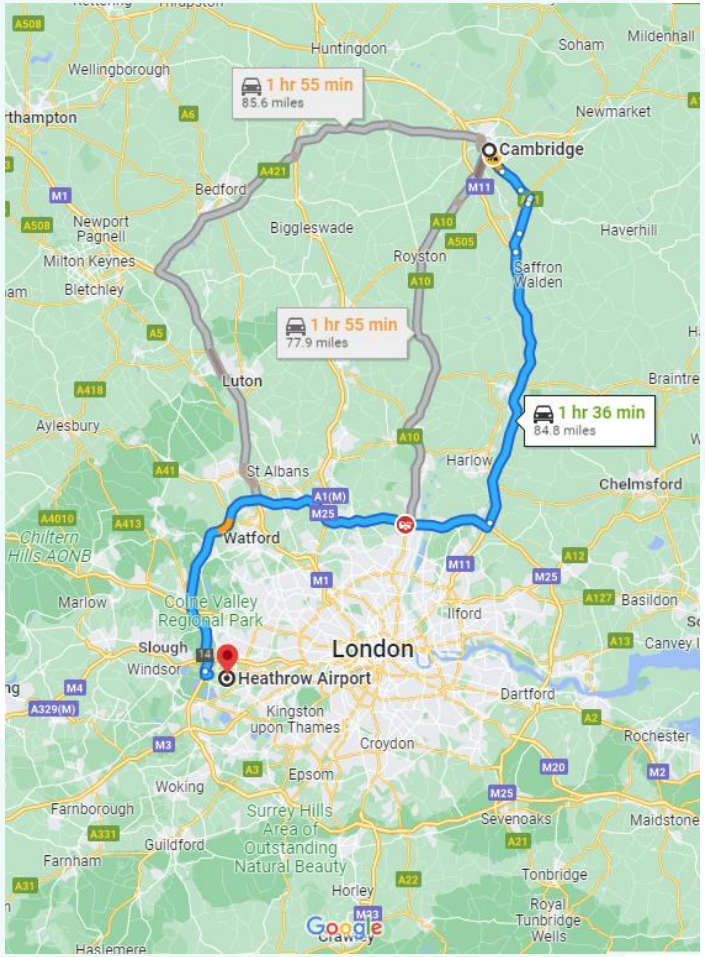
- Protectiveness: 100%
- Utility: 62%

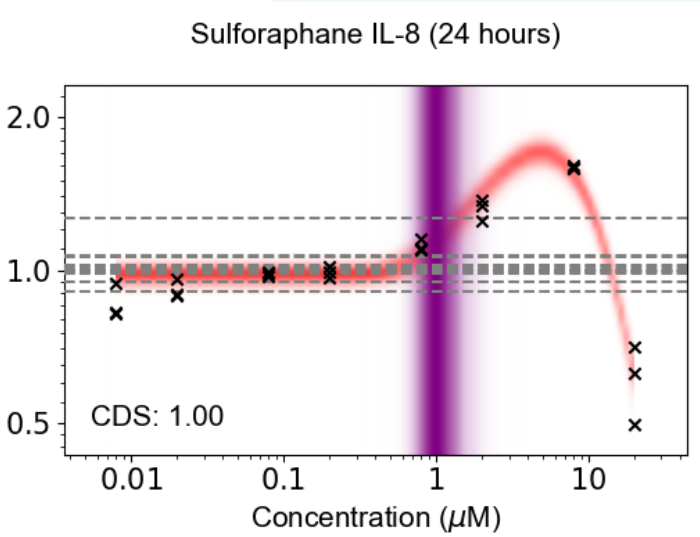
Next step for the toolbox – the full evaluation

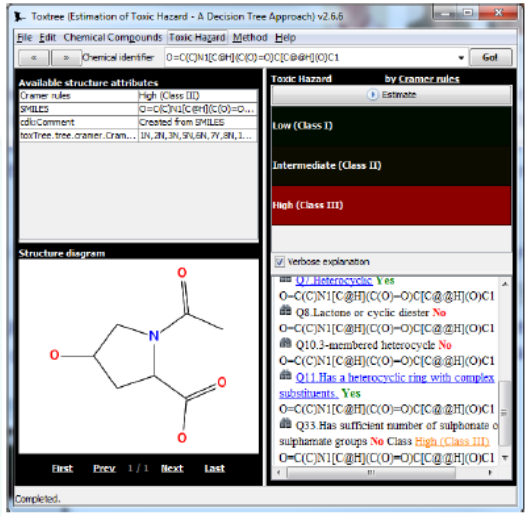


- Planning to extend evaluation to ~40 chemicals with ~60 associated **high risk** and **low risk** exposure scenarios.
- Also in collaboration with US-EPA, expanding range of NAMs
- Adopt **iterative approach** to evaluating and then identifying potential improvements to the toolbox.
- Use of concepts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

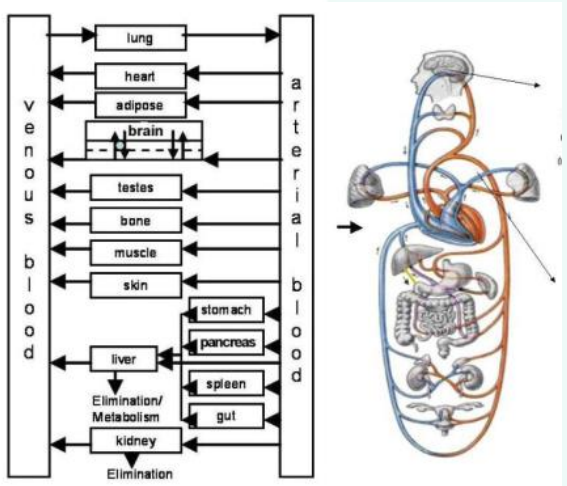
Thinking about the future...







ToxTree



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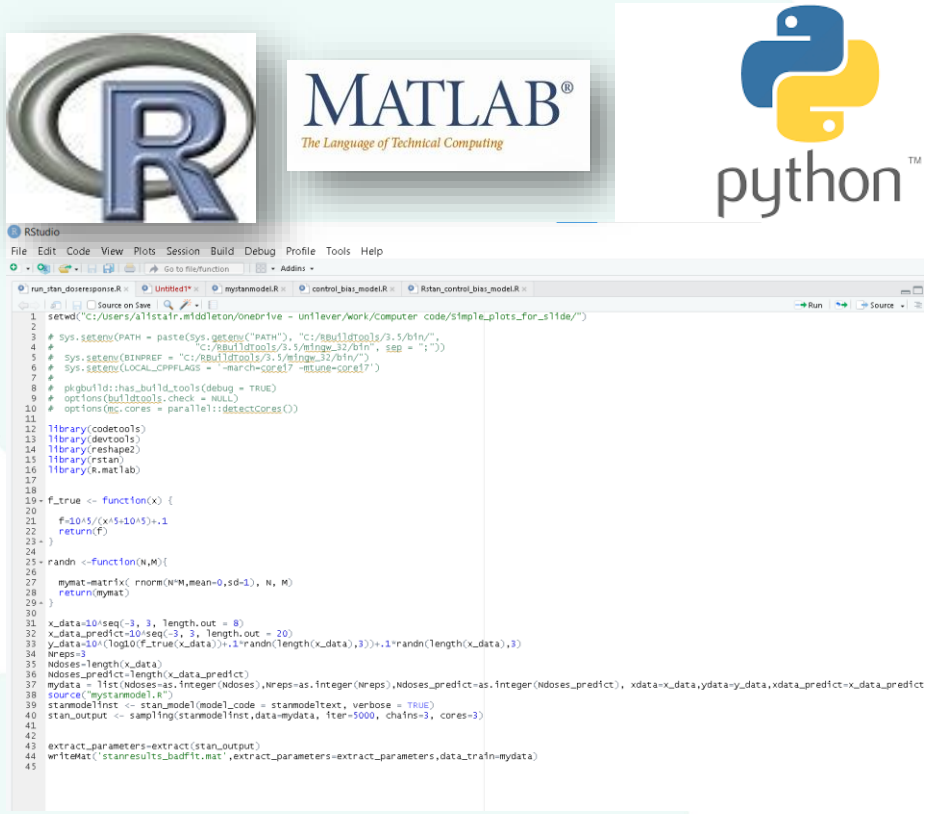
Getting started with computational approaches...

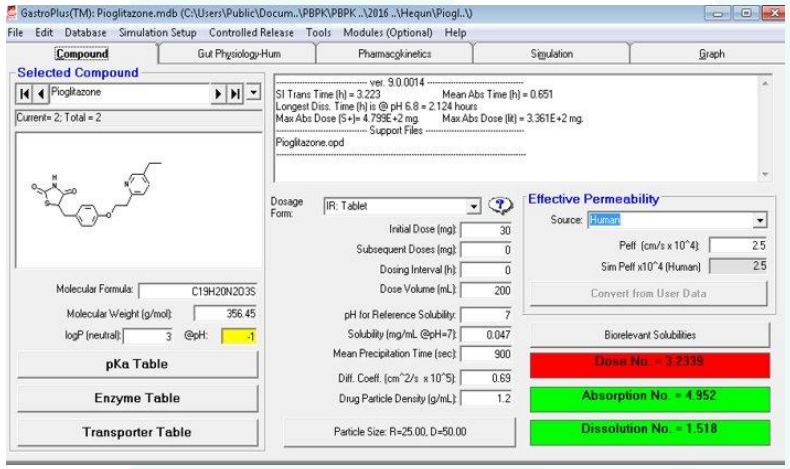
Learning to code vs using existing tools

Programming



Graphical user interfaces

PBK software



SIMCYP real solutions from virtual populations

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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/

Unilever

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