Evaluating New Approach Methodologies for Consumer-Based Risk Assessment: Challenges and Future Perspectives

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Can we safely use **x**% of ingredient **y** in product **z**?

Principles of Next generation risk assessment (NGRA) for consumer safety

*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***

ICCR
9 principles of NGRA

Main overriding principles:
The overall goal is a human safety risk assessment
The assessment is exposure led
The assessment is hypothesis driven
The assessment is designed to prevent harm
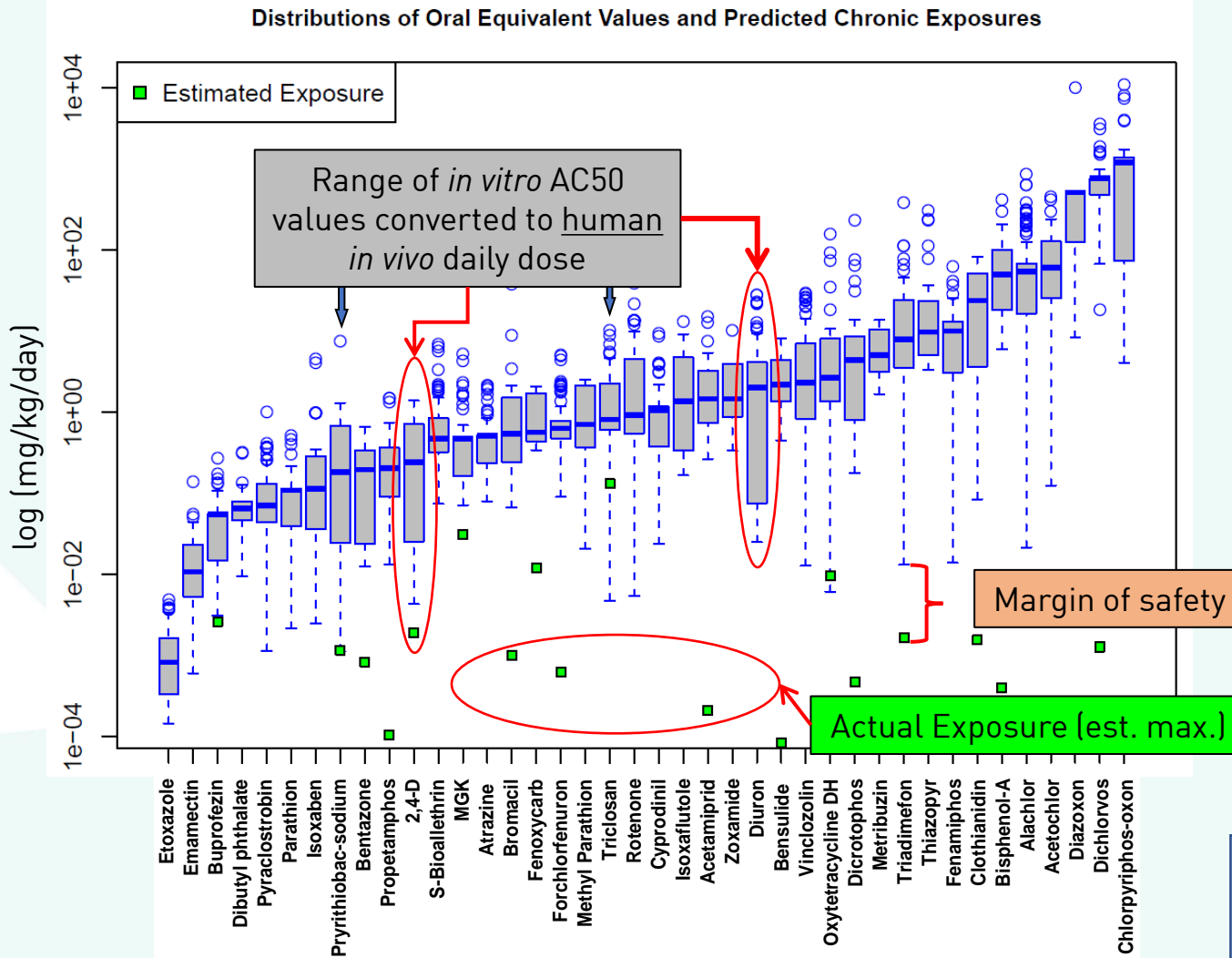
Principles describe how a NGRA should be conducted:
Following an appropriate appraisal of existing information
Using a tiered and iterative approach
Using robust and relevant methods and strategies

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

ICCR
International Cooperation
on Cosmetics Regulation

Unilever

NGRA: The assessment is designed to prevent harm

Distributions of Oral Equivalent Values and Predicted Chronic Exposures

The philosophy behind this type of risk assessment aimed at preventing harm is **based on the premise of "Protection not Prediction".**

The hypothesis underpinning this type of NGRA is that **if there is no bioactivity observed at consumer-relevant concentrations, there can be no adverse health effects.**
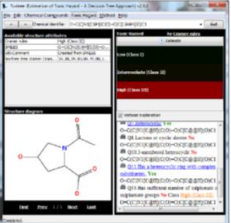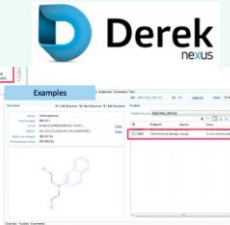
Slide from Dr Rusty Thomas, EPA, with thanks

Rotroff, *et al.* Tox.Sci 2010

From NAMs to decision making

In-vitro screening assays

In-silico tools

ToxTree

Meteor nexus

Derek nexus

OECD QSAR TOOLBOX

Receptor-binding assays

Phenotypic profiling

Cellular stress panel

e.g. AR-CALUX® assay to measure androgen receptor activity

Doxorubicin Mitochondrial mass 6 hours

CDS: 1.00

- Flutamide (DHT EC50)
- Flutamide (DHT 100xEC50)
- Test Substance (DHT EC50)
- Test Substance (DHT 100xEC50)

Live cell imaging

Increased expression of fluorescently tagged Srxn1 (green) with increased dose of Dietheyl maleate

Exposure models

PBK-models

Free concentration

Micro-physiological systems

Emulate

Lower vascular channel

Upper parenchymal channel

MIMETAS
the organ-on-a-chip company

TY10 microvessel   ECM gel   hAst & hBPCT

Unilever
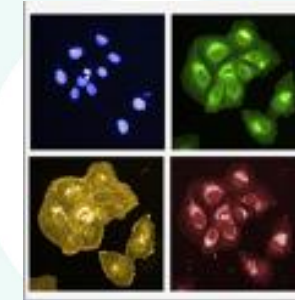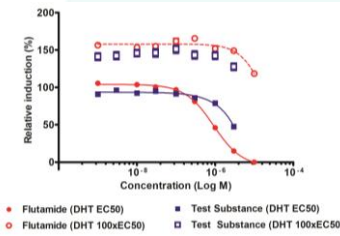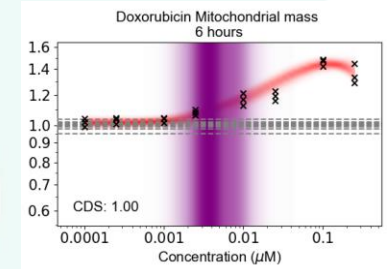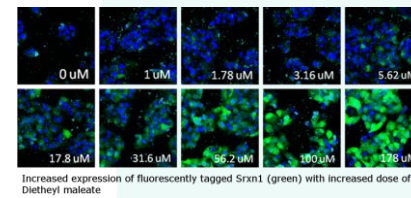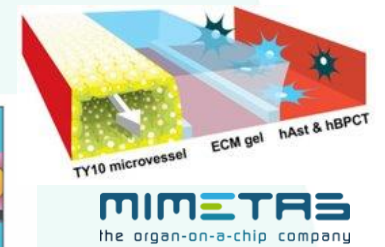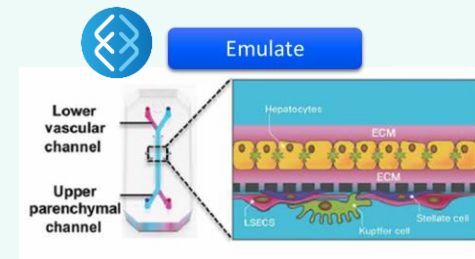
A case study approach – human health safety assessment required for...

0.1% COUMARIN IN FACE CREAM FOR EU MARKET

Assumed that:

- Coumarin was 100% pure

- no *in vivo* data was available such as animal data, History of Safe Use (HoSU) info. or Clinical data

- no use of animal data in Read Across

- *In silico* alerts known to be based on animal or *in vivo* data or on the structure of Coumarin itself were excluded
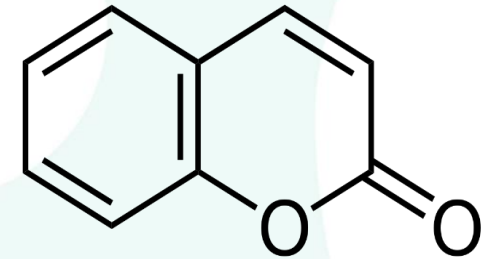
Derivation of in vitro PoD across multiple cell models (HepG2, NHEK and MCF7) & refinement with HepaRG 2D and 3D & metabolism studies
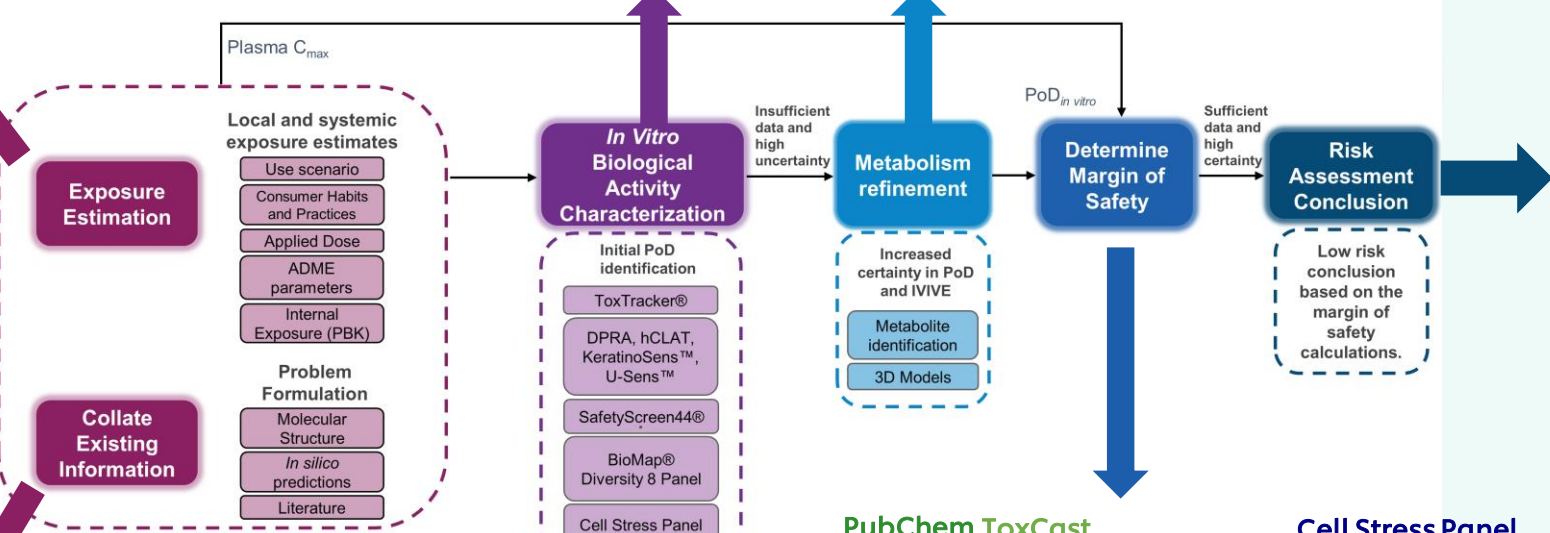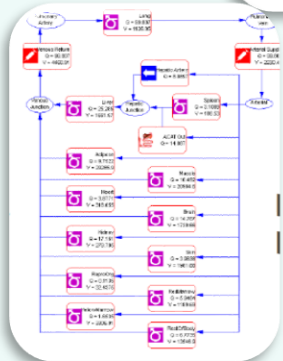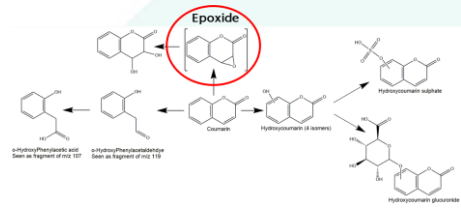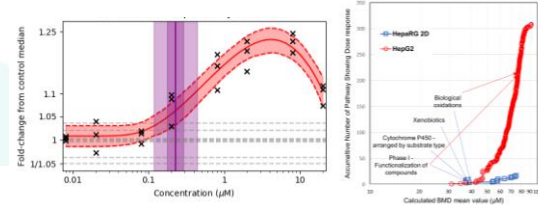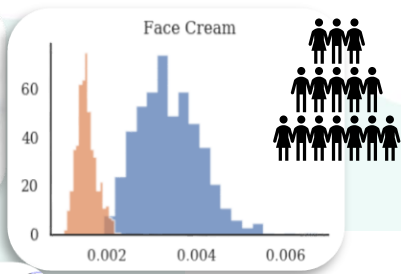
In this case study:

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

Face Cream

Plasma C$_{max}$

Exposure Estimation

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

Problem Formulation
- Molecular Structure
- In silico predictions
- Literature

Collate Existing Information

In Vitro Biological Activity Characterization

Initial PoD identification
- ToxTracker®
- DPRA, hCLAT, KeratinoSens™, U-Sens™
- SafetyScreen44®
- BioMap® Diversity 8 Panel
- Cell Stress Panel
- HTTr – TempO-Seq

Insufficient data and high uncertainty

Metabolism refinement

Increased certainty in PoD and IVIVE
- Metabolite identification
- 3D Models

PoD$_{in vitro}$

Sufficient data and high certainty

Determine Margin of Safety

Risk Assessment Conclusion

Low risk conclusion based on the margin of safety calculations.

Epoxide

QSAR TOOLBOX

OECD

SOT Society of Toxicology   ToxSci 20 Years

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

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

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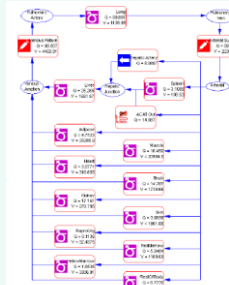and Paul J. Russell†

Derek nexus

Meteor nexus

Tox21/ToxCast ~700 HTS Biological Pathways Assays

EPA   iCSS ToxCast Dashboard

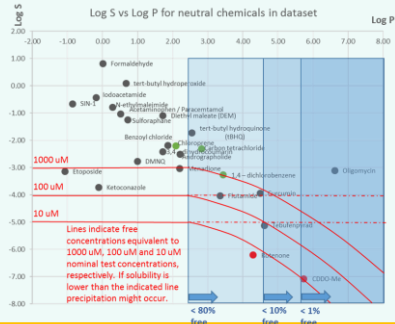**PubChem ToxCast**   **Cell Stress Panel**   **HTTr**

Unilever
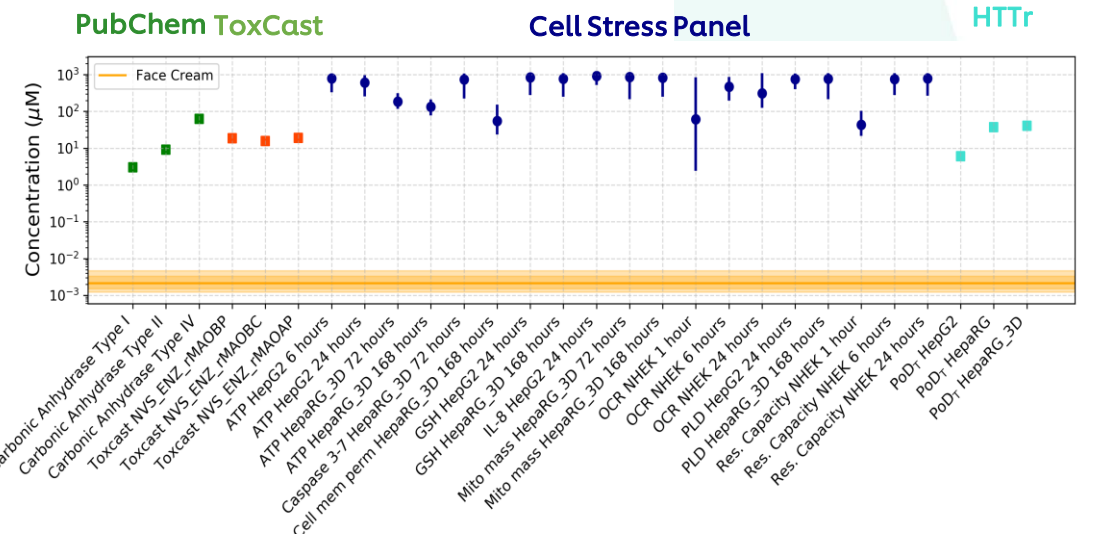
Could these NAMs provide a low-tier toolset for systemic toxicity?

PBK models　**Free concentration**　**Conc. Resp. models**　**HTTr**　**Cell stress**　**CEREP 44**
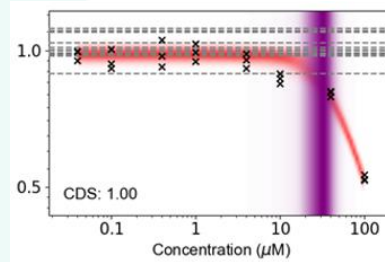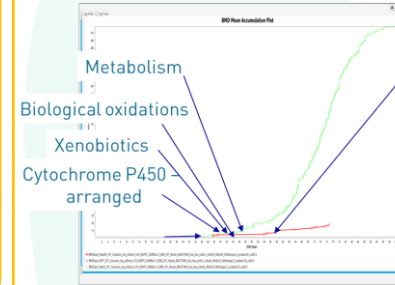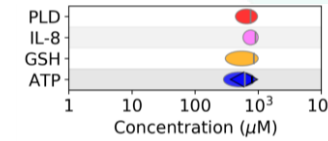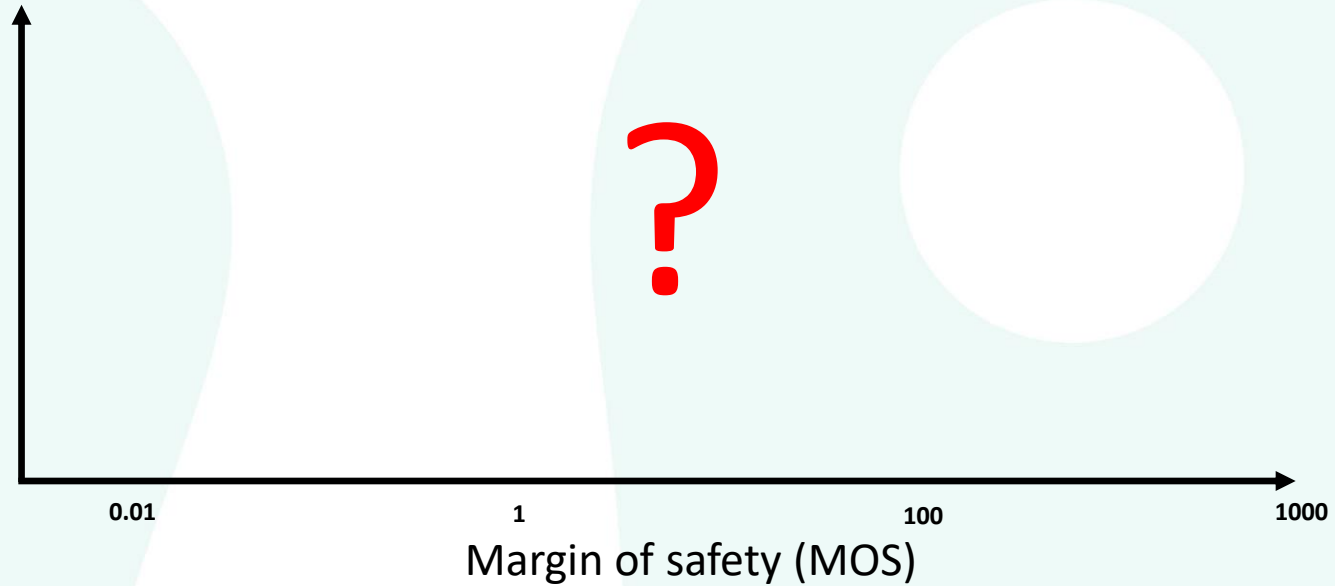

Margin of Safety estimate
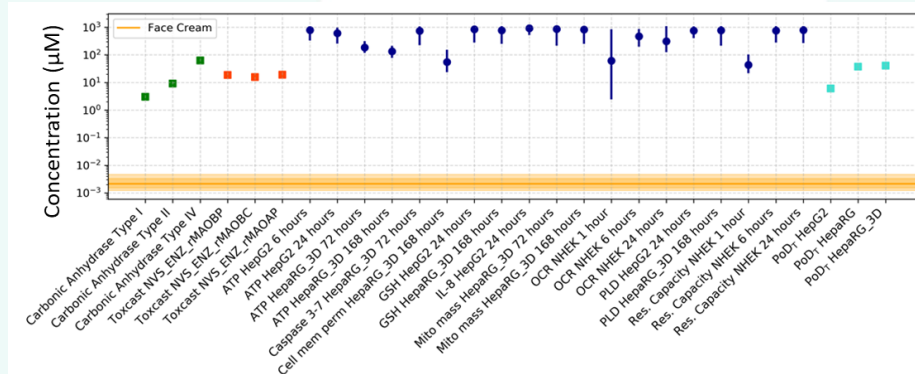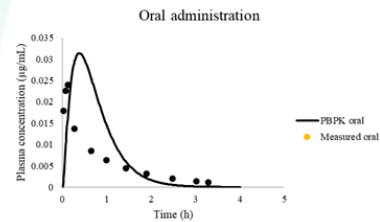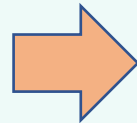

Inform safety decision

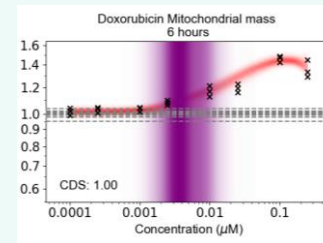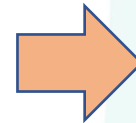# Evaluating the toolset for risk assessment: a data driven approach

Chemical exposures scenarios

🟢 'Low' risk (from consumer goods perspective) – e.g. foods, cosmetics
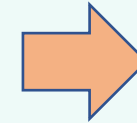
🔴 'High' risk (from consumer goods perspective) – e.g. drugs

Margin of safety (MOS)

Define typical use-case scenarios benchmark chemical-exposures ➡️ **PBK models of systemic exposure** ➡️ **Calculate the PoDs** ➡️ **Calculate the margin of safety**

Can the toolset successfully **distinguish between low and high risk** chemical exposure scenarios up to a certain MOS?

Evaluating the toolset for risk assessment: a data driven approach
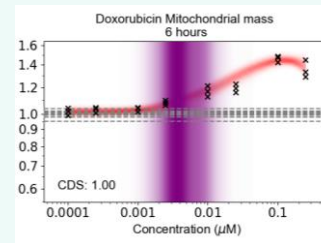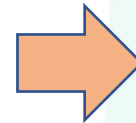
Chemical exposures scenarios

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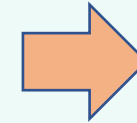🔴 'High' risk (from consumer goods perspective) – e.g. drugs

Margin of safety (MOS)

Define typical use-case scenarios benchmark chemical-exposures

➡️

PBK models of systemic exposure

➡️

Calculate the PoDs

➡️
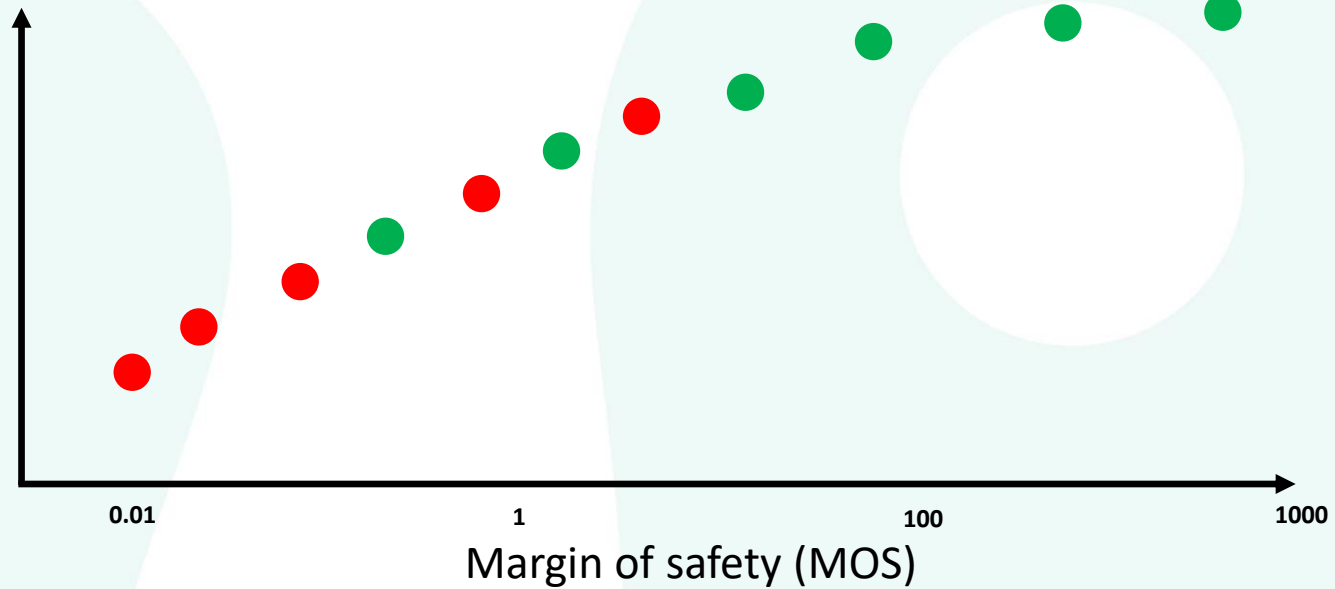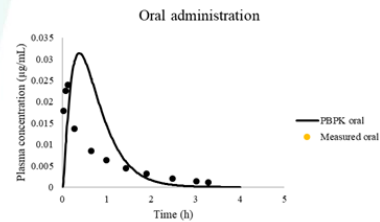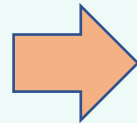
Calculate the margin of safety

Can the toolset successfully **distinguish between low and high risk** chemical exposure scenarios up to a certain MOS?

Characterising potential non-specific modes of toxicity using a cellular stress panel
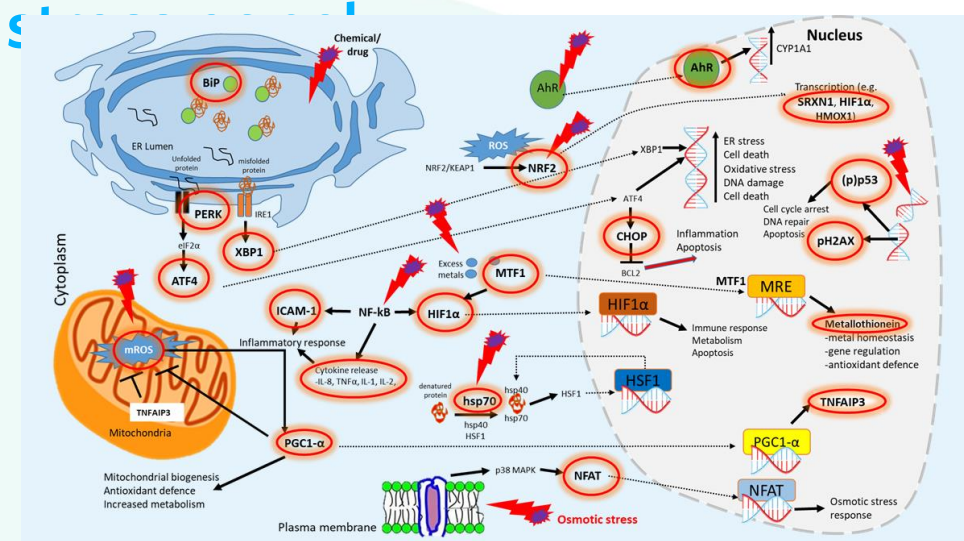

Image kindly provided by Paul Walker (Cyprotex)

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

- 13 test substances, each with an associated exposure scenario, e.g:
  - (Low risk) Niacinamide as a cosmetic ingredient, exposure level based on tolerable daily intake.
  - (High risk) Doxorubicin as a chemotherapy drug, exposure based on therapeutic dose.
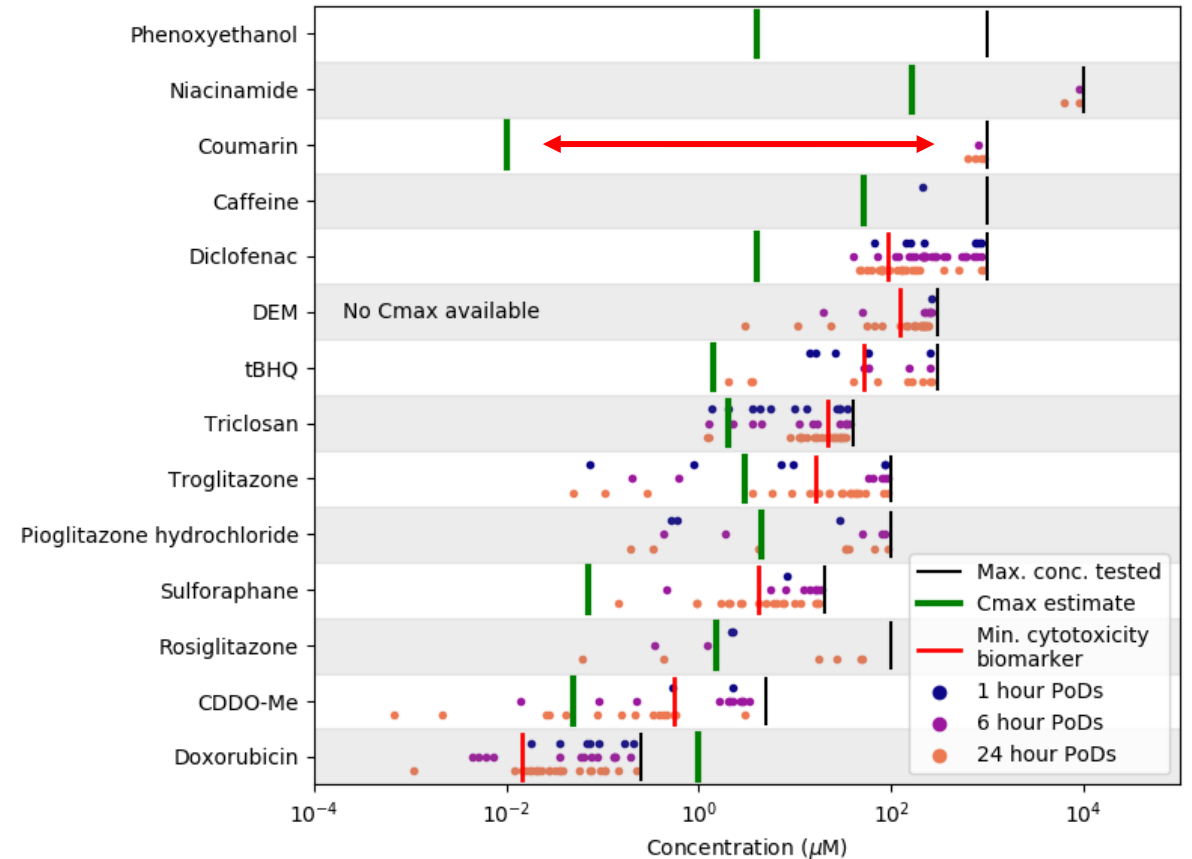
Hatherell S, Baltazar MT, Reynolds J, et al. Identifying and Characterizing Stress Pathways of Concern for Consumer Safety in Next-Generation Risk Assessment. *Toxicol Sci*. 2020;176(1):11-33. doi:10.1093/toxsci/kfaa054

Margin of safety vs chemical-exposure risk category using the cell stress panel

Exposure scenario adopted for chemical is 'low risk' (from consumer goods perspective)
- Niacinamide (food, cosmetics)
- Caffeine (beverages, cosmetics)
- Phenoxyethanol (cosmetics)
- Sulforaphane (food)
- tBHQ (antioxidant)
- Triclosan (antimicrobial)

Exposure scenario adopted for chemical is 'high risk' (from consumer goods perspective).
- CDDO-Me (drug)
- DEM (industrial chemical)
- Doxorubicin (drug)
- Diclofenac (drug)
- Troglitazone (drug)
- Pioglitazone (drug)
- Rosiglitazone (drug)
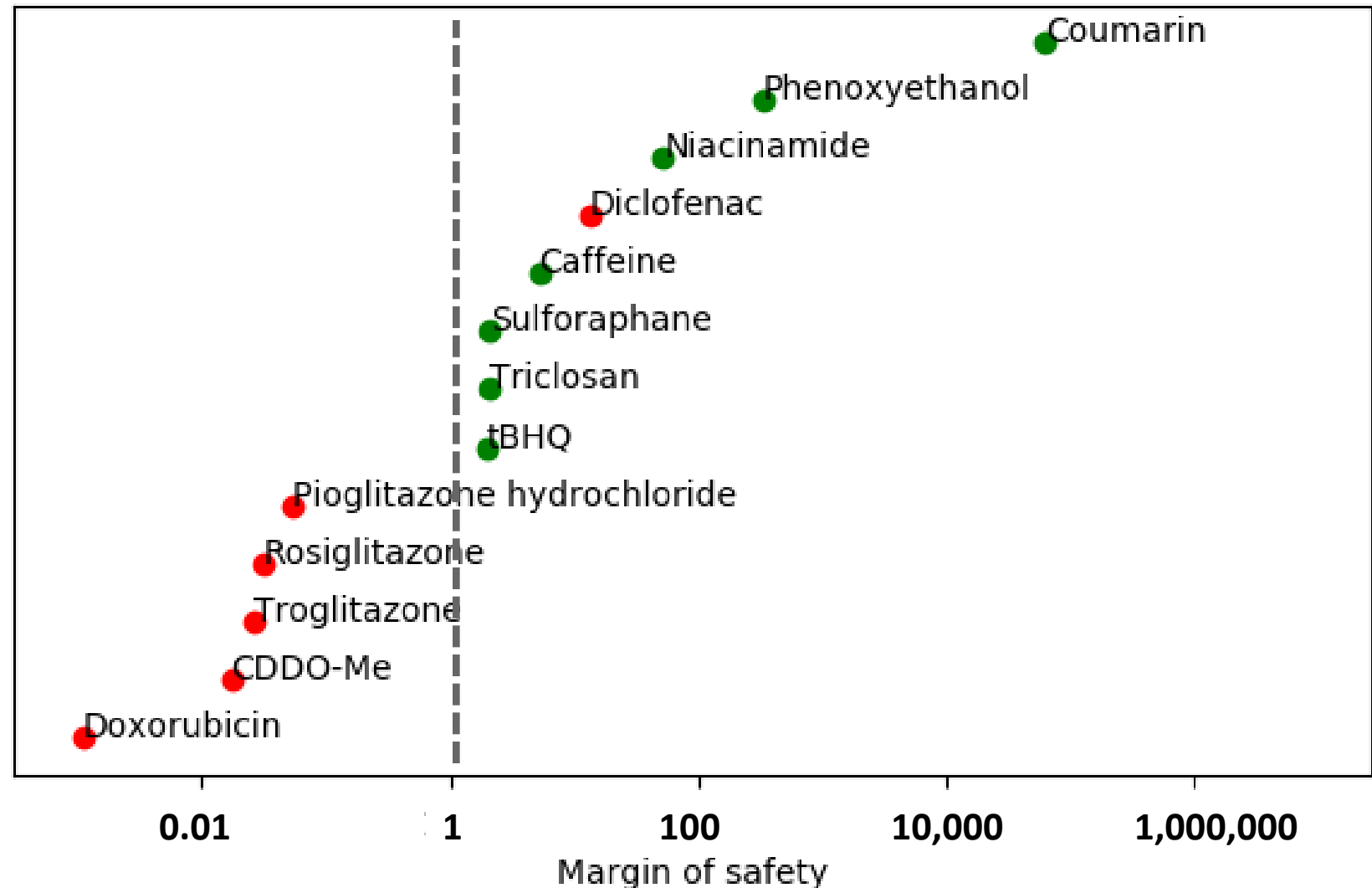
An evaluation strategy

- Identify 40+ appropriate chemical-exposure scenarios
- Run the chemicals through the tools as though de-novo compounds.
- Single timepoint (24-hour)
- Single exposure
- Three cell lines for the HTTr (HepG2, HepaRG, MCF-7).

Can distinguish high risk and low risk based on MOS

Margin of safety (MOS)

Chemical exposures scenarios

- 'Low' risk (from consumer goods perspective) – e.g. foods, cosmetics
- 'High' risk (from consumer goods perspective) – e.g. drugs
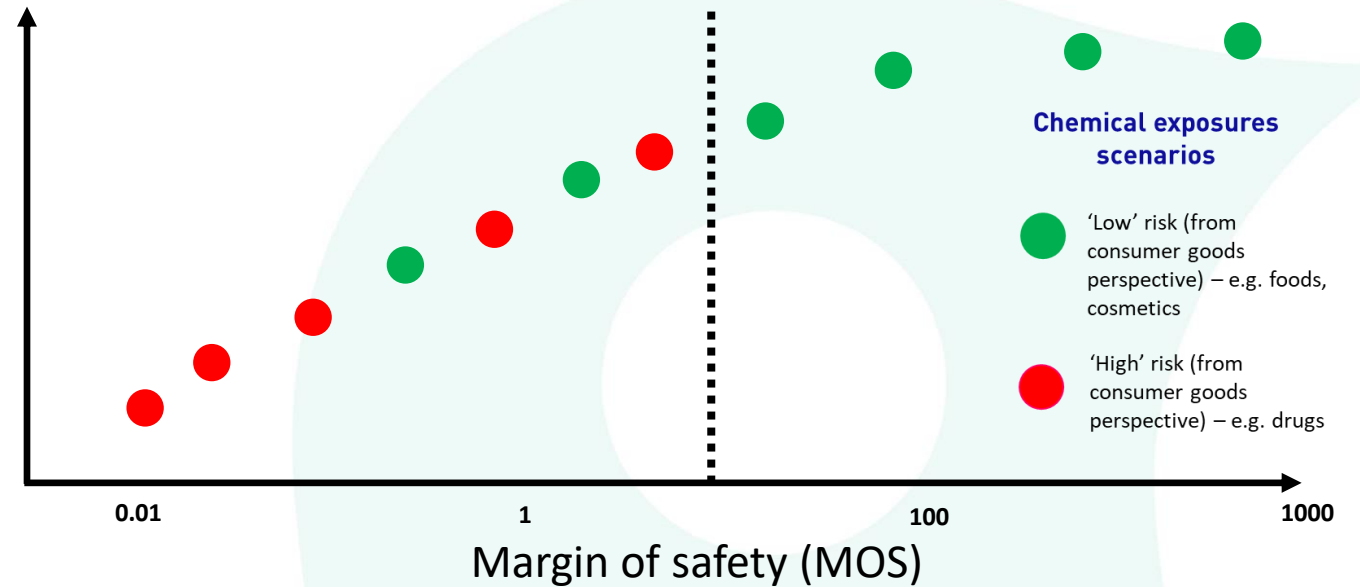
Can not distinguish high risk and low risk based on MOS

Margin of safety (MOS)
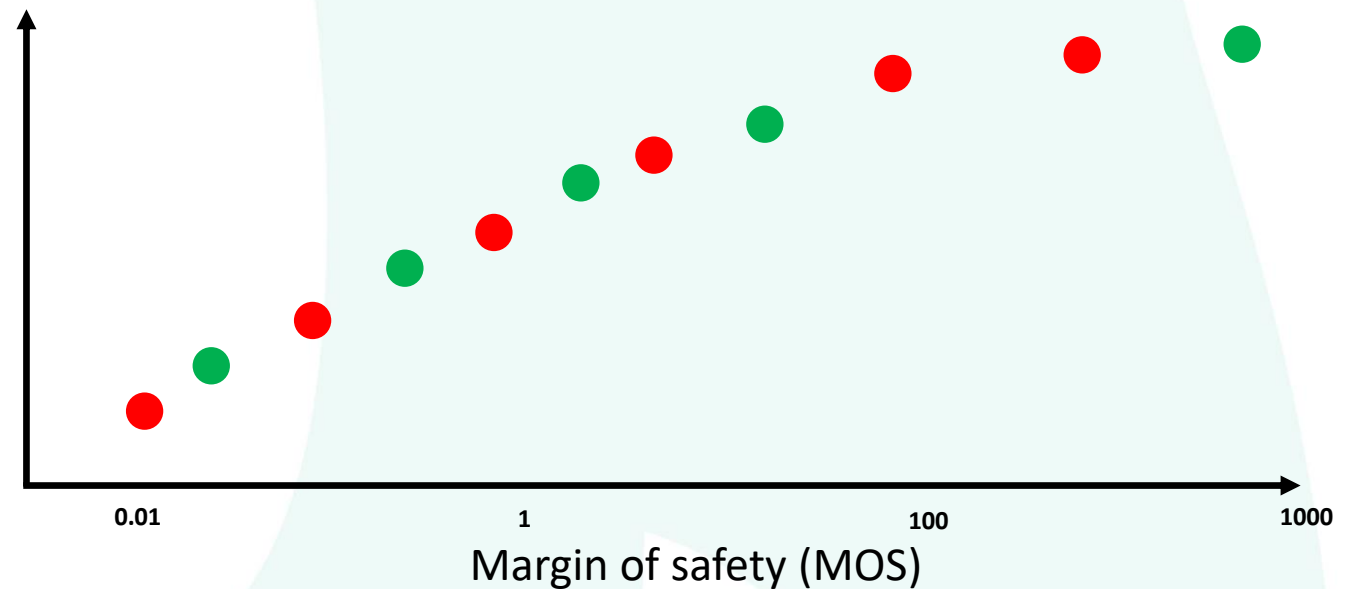
Challenges and potential solutions to implementing the strategy

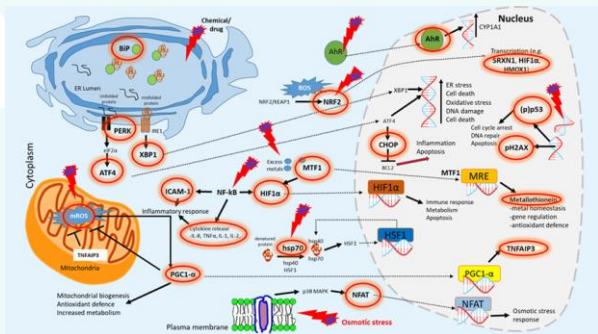## Identification of appropriate chemical-exposures

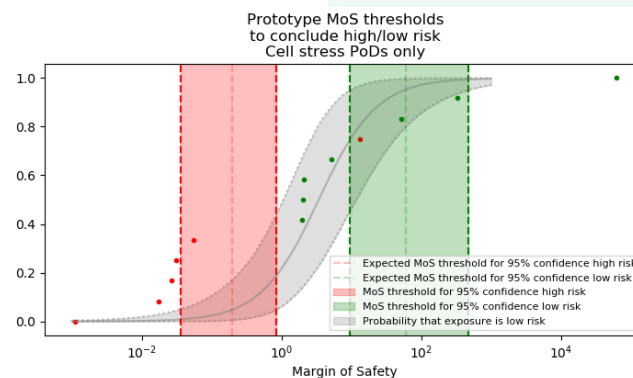## Uncertainty in exposure estimates (inc metabolism)
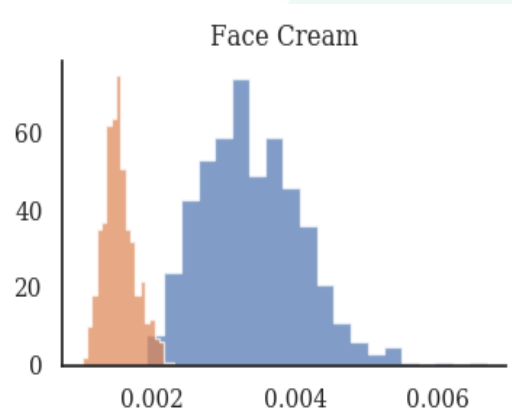
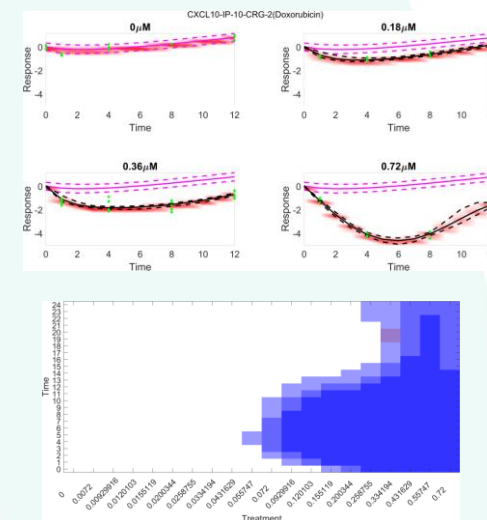## Uncertainty in PoD estimates and free concentration
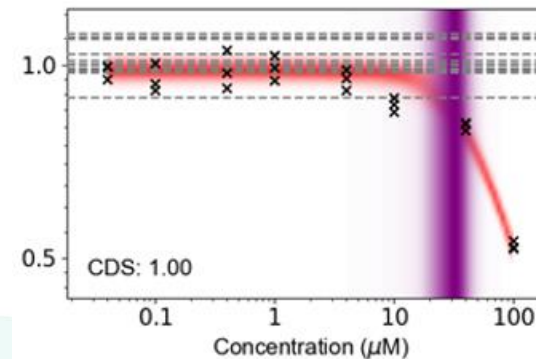
Sufficient biological coverage (assays and cell models)

Robust decision-making based on the MOS

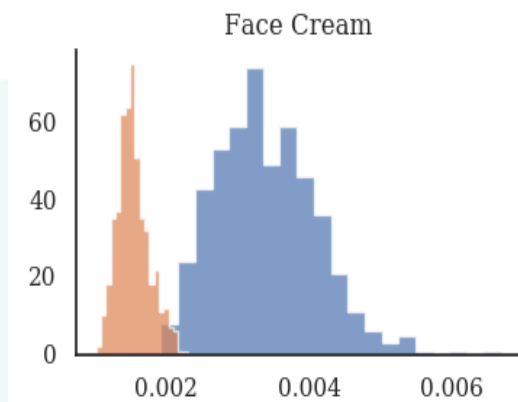## Time-dependence of cellular responses

Moxon TE, Li H, Lee MY, et al. Application of physiologically based kinetic (PBK) modelling in the next generation risk assessment of dermally applied consumer products. *Toxicol In Vitro*. 2020;63:104746. doi:10.1016/j.tiv.2019.104746

What we're doing to address these challenges (1/3)

Identification of appropriate chemical-exposures

- **Systematic selection of different chemicals with defined human-use scenarios (cosmetics, drugs, etc)**
- **Use of both automated and manual data extraction approaches**
- **Address potential statistical bias through randomised selection of chemical-exposure scenarios together with hand-selected scenarios.**

Uncertainty in exposure estimates (how 'wrong' are the PBK models?)

- **Evaluation of 'how wrong' PBK models can be by comparing human Cmax/AUC data to model predictions**
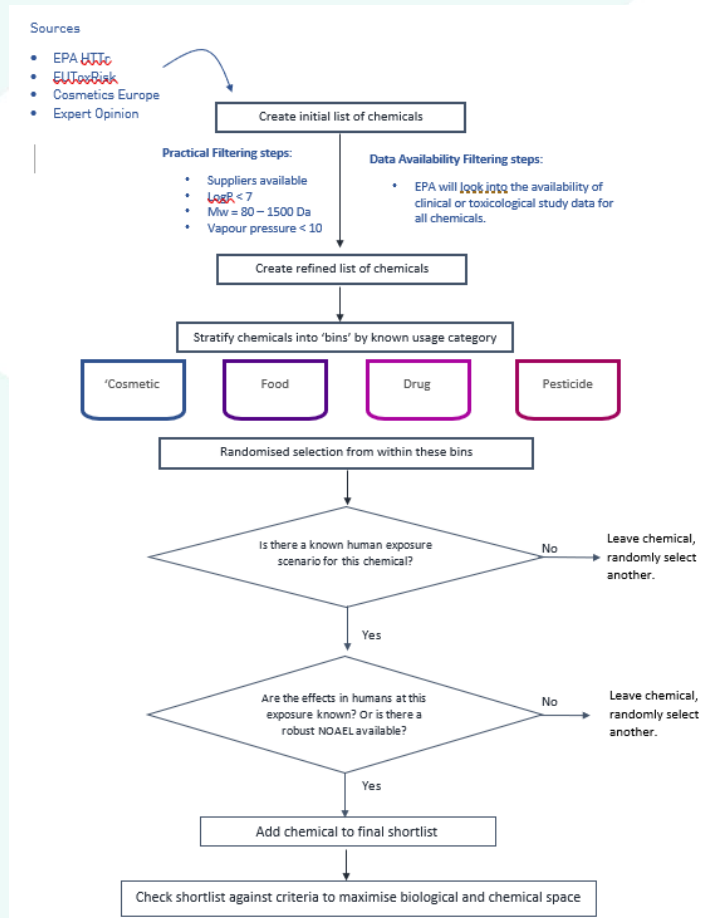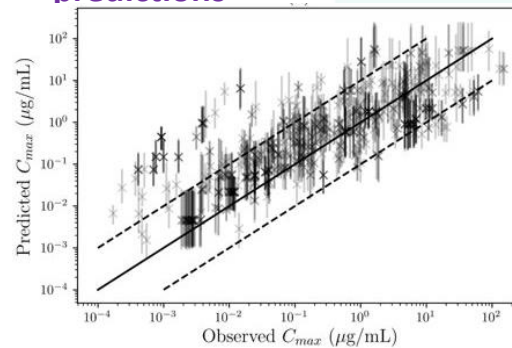
What we're doing to address these challenges (2/3)

Uncertainty in PoD estimates
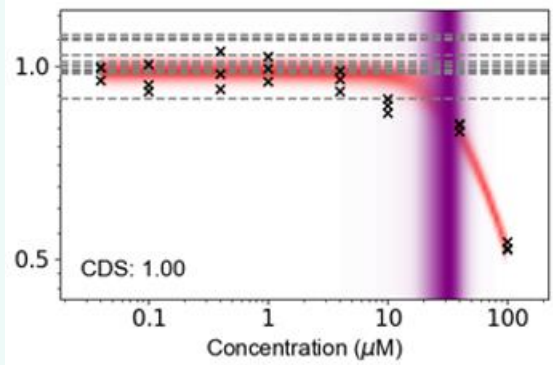
PoD variability across cell models and replicates
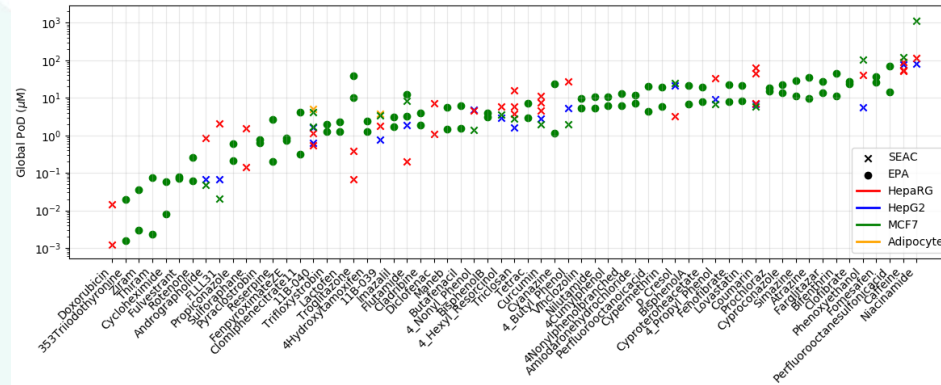
Plate effect example
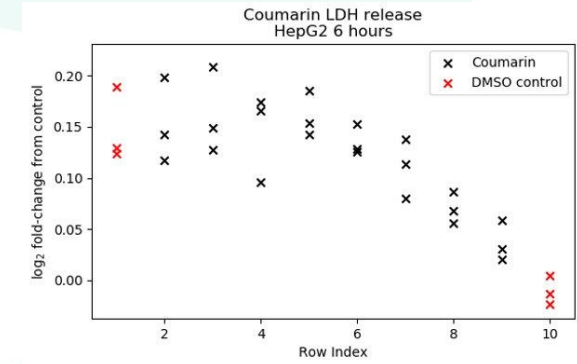
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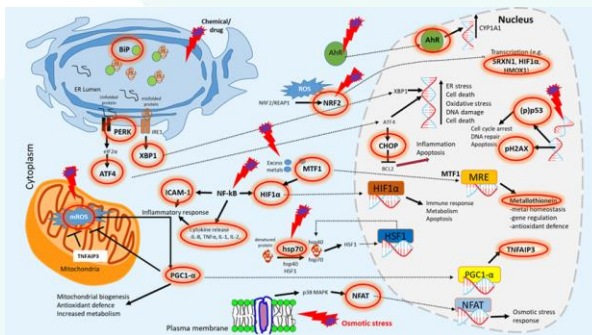
- **Optimising experimental design of our assays (number of replicates, plate layout, appropriate controls etc)**
- **Compare different PoD calculation approaches (BMDexpress etc)**
- **Analysing biological pathway coverage across large numbers of compounds and cell types.**
- **Evaluating other broad-spectrum assays (e.g. phenotypic profiling).**

Robust decision-making based on the MOS
using e.g. Bayesian logistic regression

Prototype MoS thresholds
to conclude high/low risk
Cell stress PoDs only

Legend:
- Expected MoS threshold for 95% confidence high risk
- Expected MoS threshold for 95% confidence low risk
- MoS threshold for 95% confidence high risk
- MoS threshold for 95% confidence low risk
- Probability that exposure is low risk

Margin of Safety

Margin (MOS)

Margin of safety (MOS)
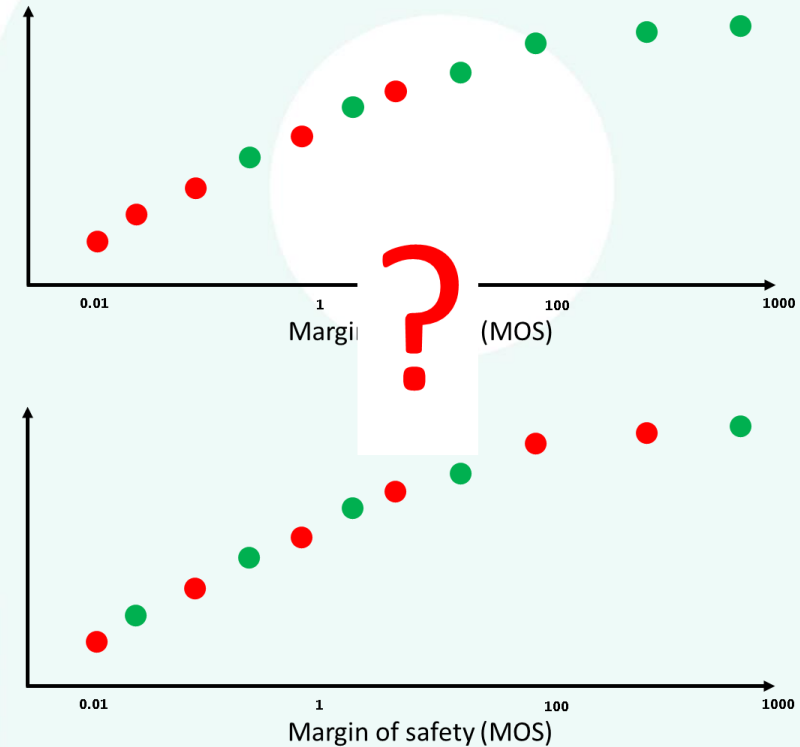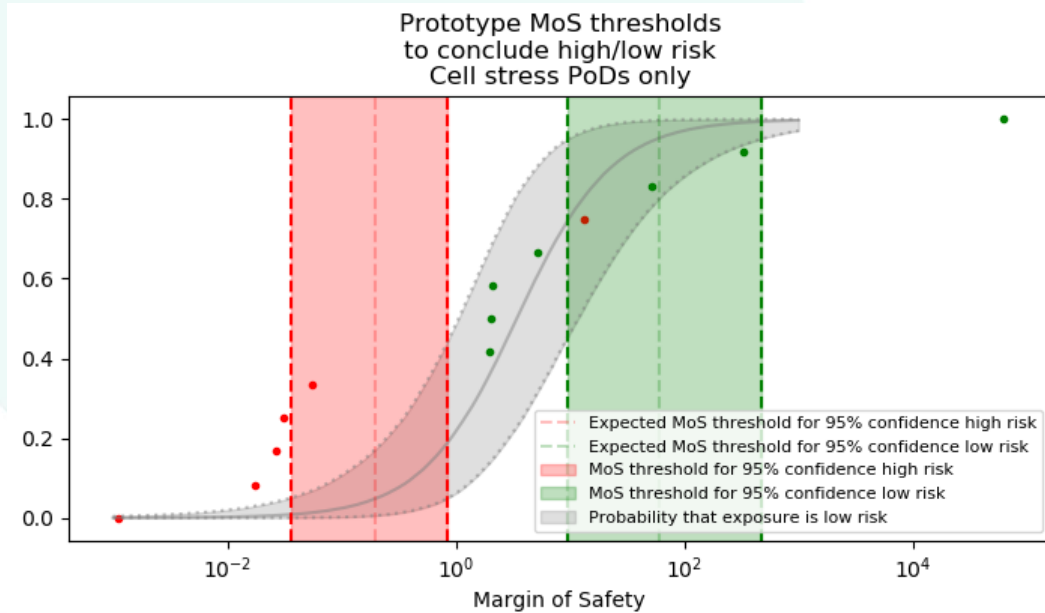
Using the toolbox data, deploy probabilistic models that quantify the (un)certainty that a given exposure scenario is low-risk based on the margin-of-safety.

Concluding remarks

1. In the context of risk assessment, NAMs must be primarily evaluated according to the decisions that will be made when using them.

2. Selecting and identifying appropriate chemical-exposure scenarios is perhaps one of the biggest challenges we face.

3. The approach described represents a low-tier toolset that could potentially cover the majority of cases. The added value of using more sophisticated tools (e.g. micro-physiological systems) is also being evaluated.

Acknowledgements

Unilever

Maria Baltazar, Tom Cull, Joe Reynolds, Beate Nicol, Mi-Young Lee, Predrag Kukic, Alexis Nathanail, Sophie Cable, Georgia Reynolds, Mona Delagrange, Tom Moxon, Hequn Li, Mabel Cotter, Jade Houghton, Andy White, Matthew Dent, Paul Carmichael, Sarah Hatherell, Sophie Malcomber, Richard Cubberley, Ruth Pendlington, Paul Russell

Cyprotex

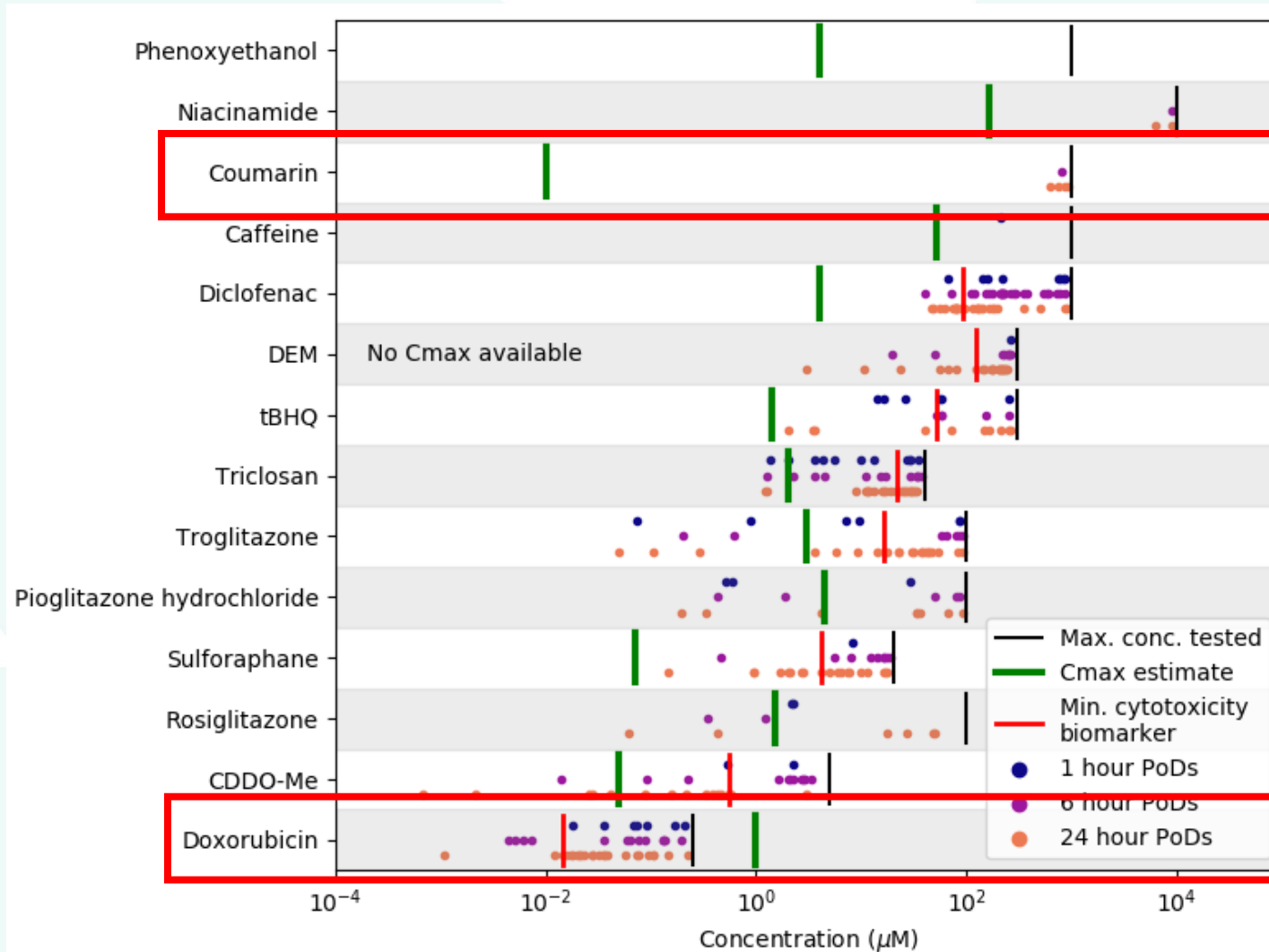Paul Walker, Stephanie Ryder, Caroline Bauch

Cambridge

Andreas Bender, Danilo Basili

EPA

Imran Shah, Richard Judson, Bryant Chambers, Josh Harrell, Logan Everett

NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: In vitro cell stress panel

Results:

Coumarin not very active in comparison to known "high risk compounds" like doxorubicin

- PoDs shown for HepG2 only

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