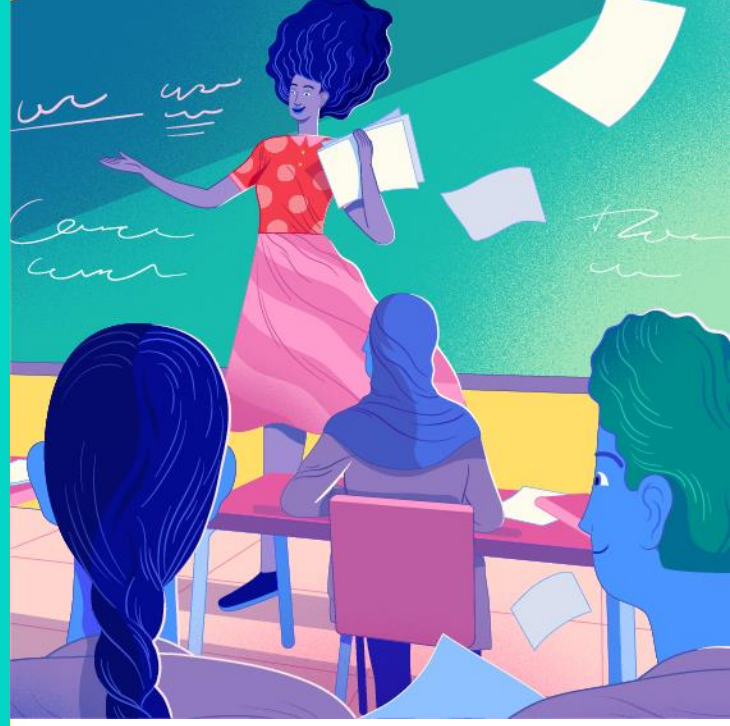
An industry perspective on strategies for integrating new approach methodologies for Next Generation Risk Assessment: coumarin as a case study

Maria Baltazar



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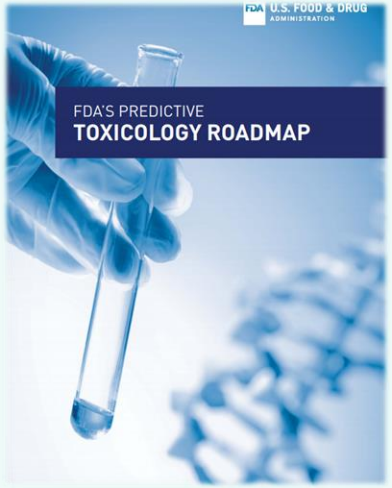
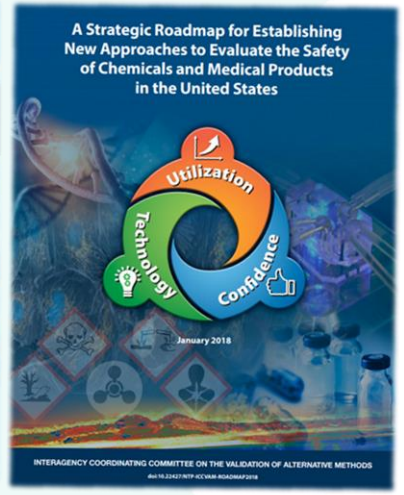
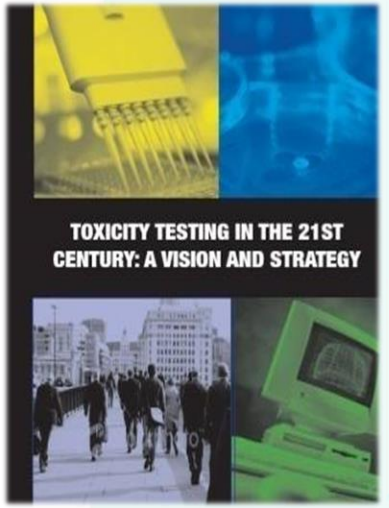
Next Generation Risk Assessment (NGRA)

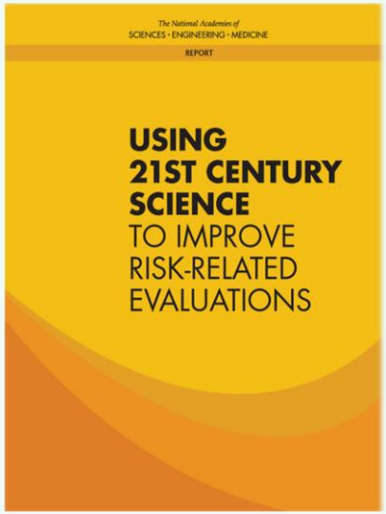
*NGRA is defined as **an exposure-led**, **hypothesis-driven** risk assessment approach that **integrates New Approach Methodologies (NAMs)** to assure **safety without the use of animal testing***

Safety without animal testing









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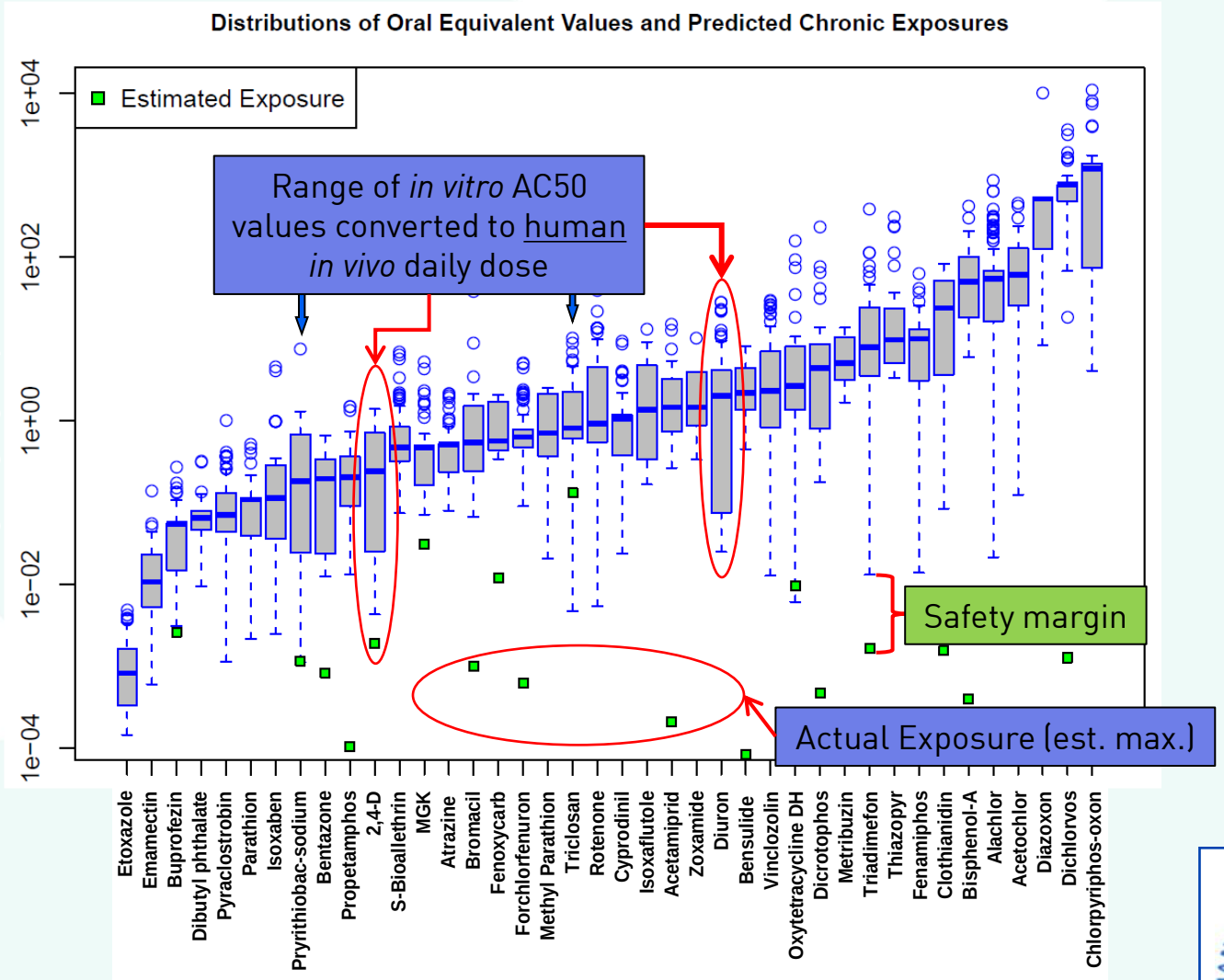
A fundamental principle of NGRA: 'Protection not prediction'



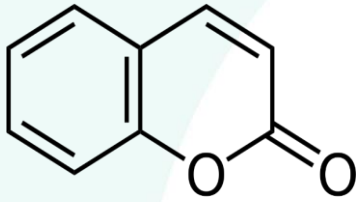
Slide from Dr Rusty Thomas, EPA, with thanks

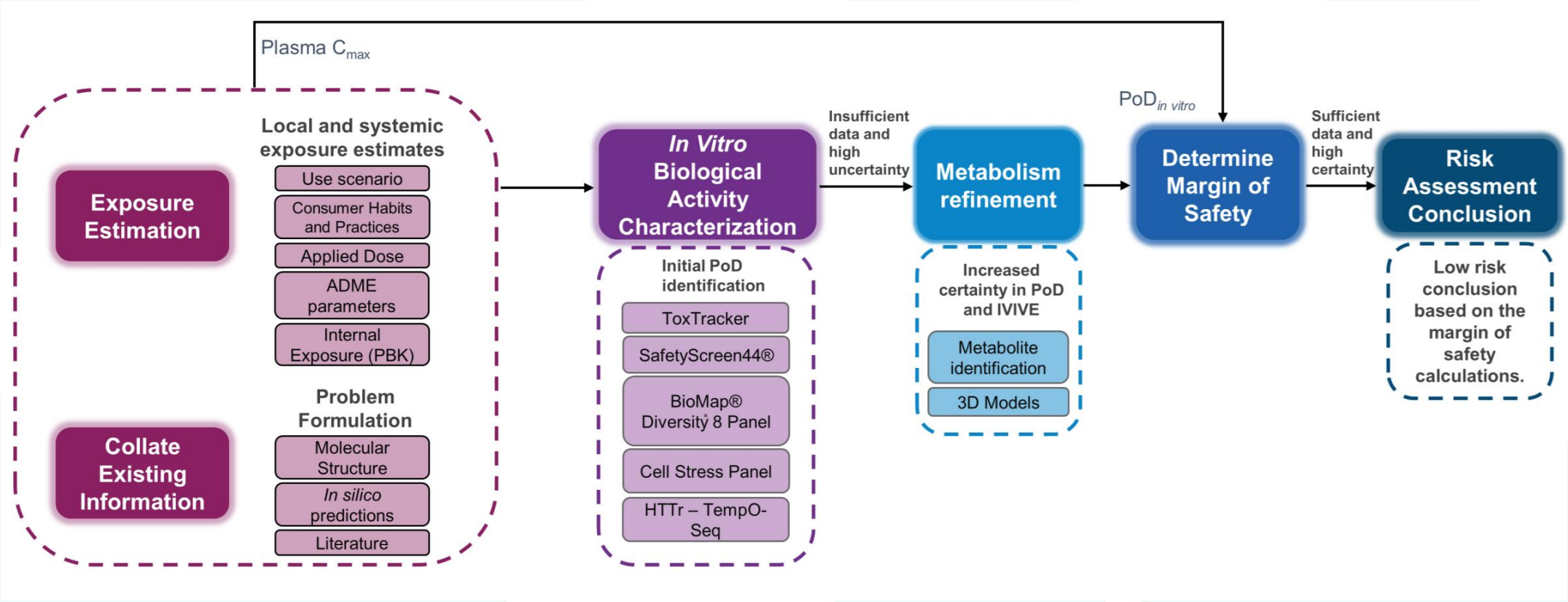
Rotroff, *et al.* Tox.Sci 2010

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.**

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

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

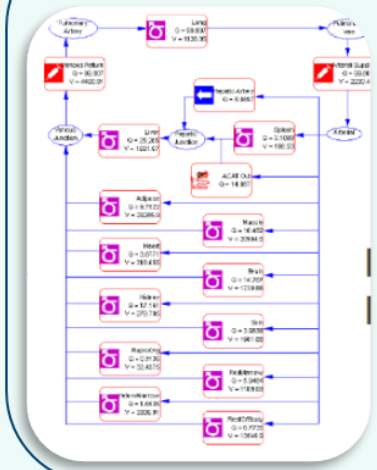


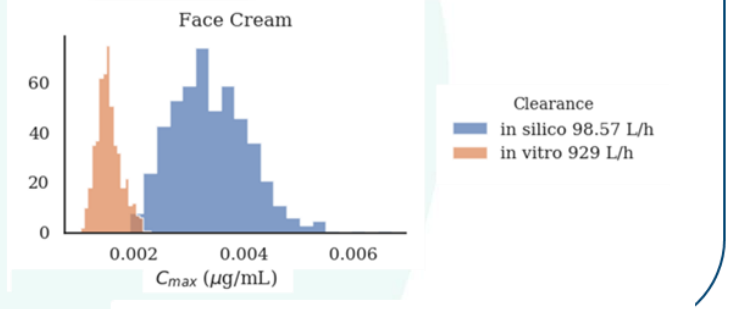


Baltazar *et al.*, (2020) *Tox Sci* Volume 176, Issue 1, 236–252

The key elements in our NGRA approach

PBK Modelling





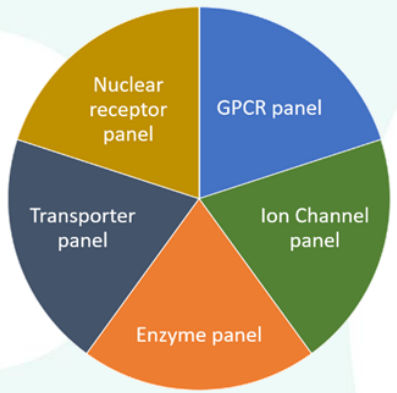
Toxicology in Vitro (2020), **63**, 104746

In vitro pharmacological profiling


PERSPECTIVES

A GUIDE TO DRUG DISCOVERY — OPINION

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

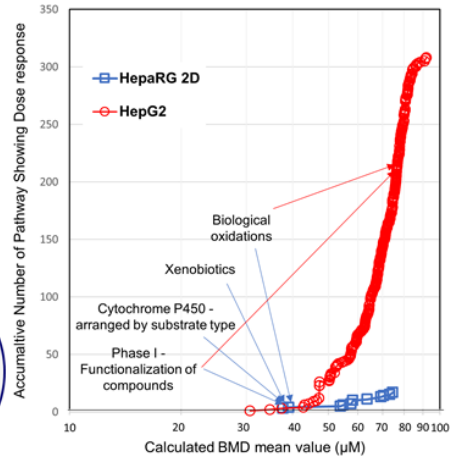


eurofins | Cerep

Transcriptomics

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

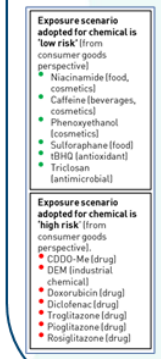
BMDExpress 2

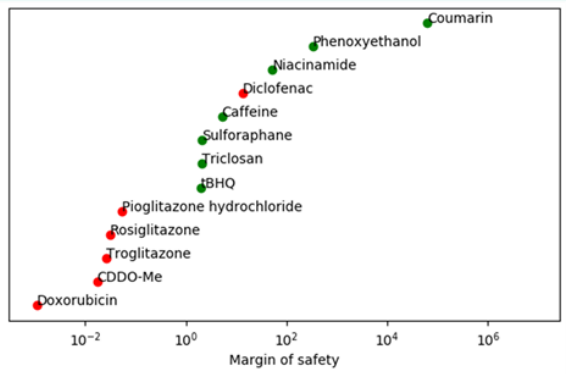


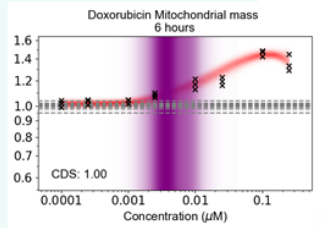
Cellular Stress Pathways

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

cyprotex AN EVOTEC COMPANY







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

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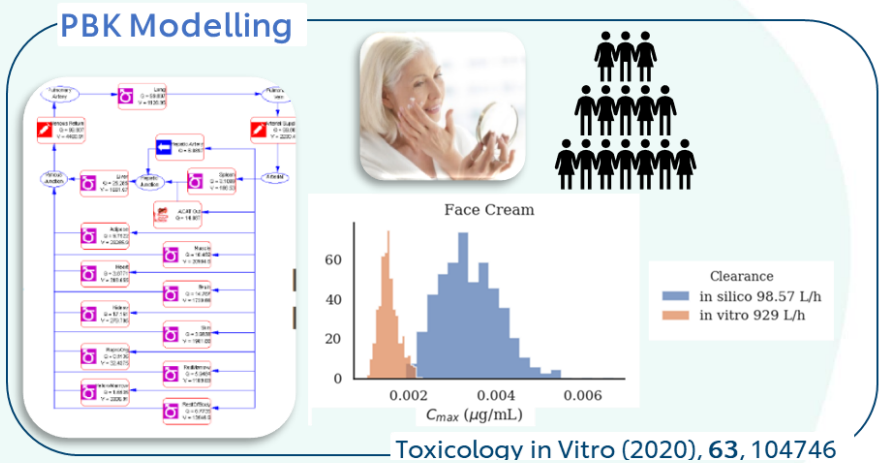
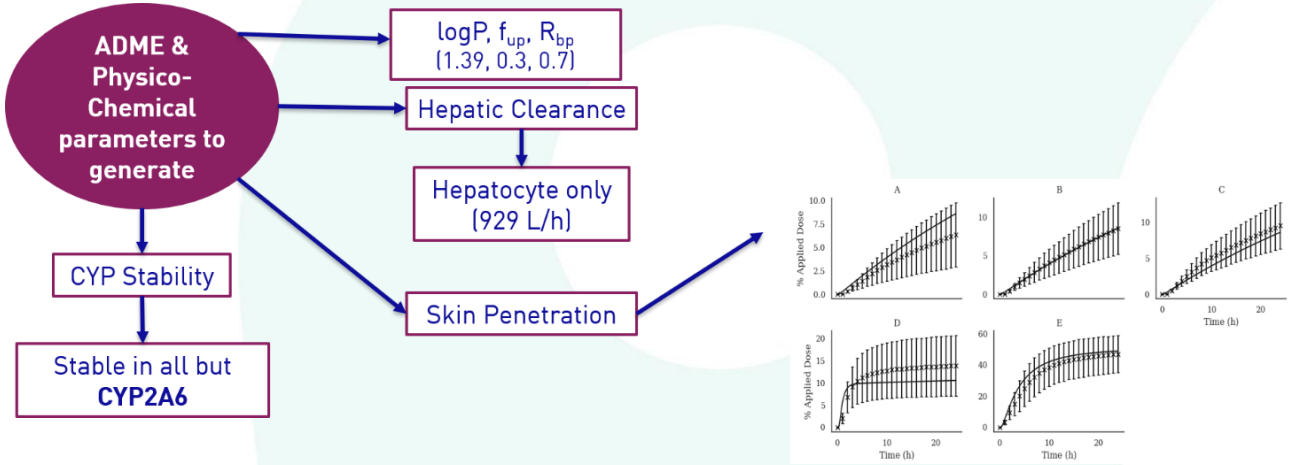
The key elements in our NGRA approach- example with the coumarin case study



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



In this case, distributions of C_{max} values were determined for both face cream and body lotion use scenarios.

The final output for coumarin shows possible distributions at two different clearance rate (*in silico* and *in vitro*) to visualise the impact this parameter can have on the predicted C_{max}

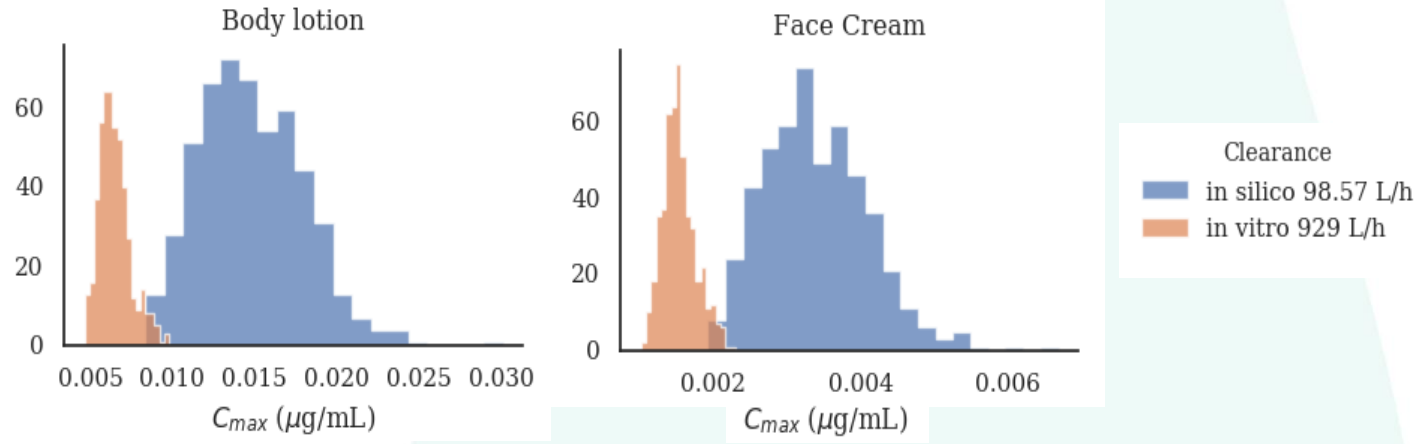
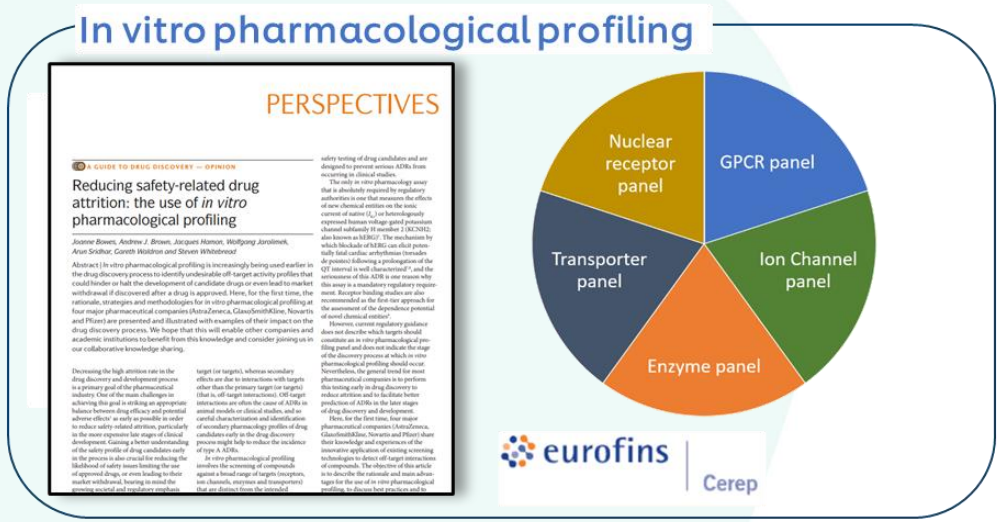


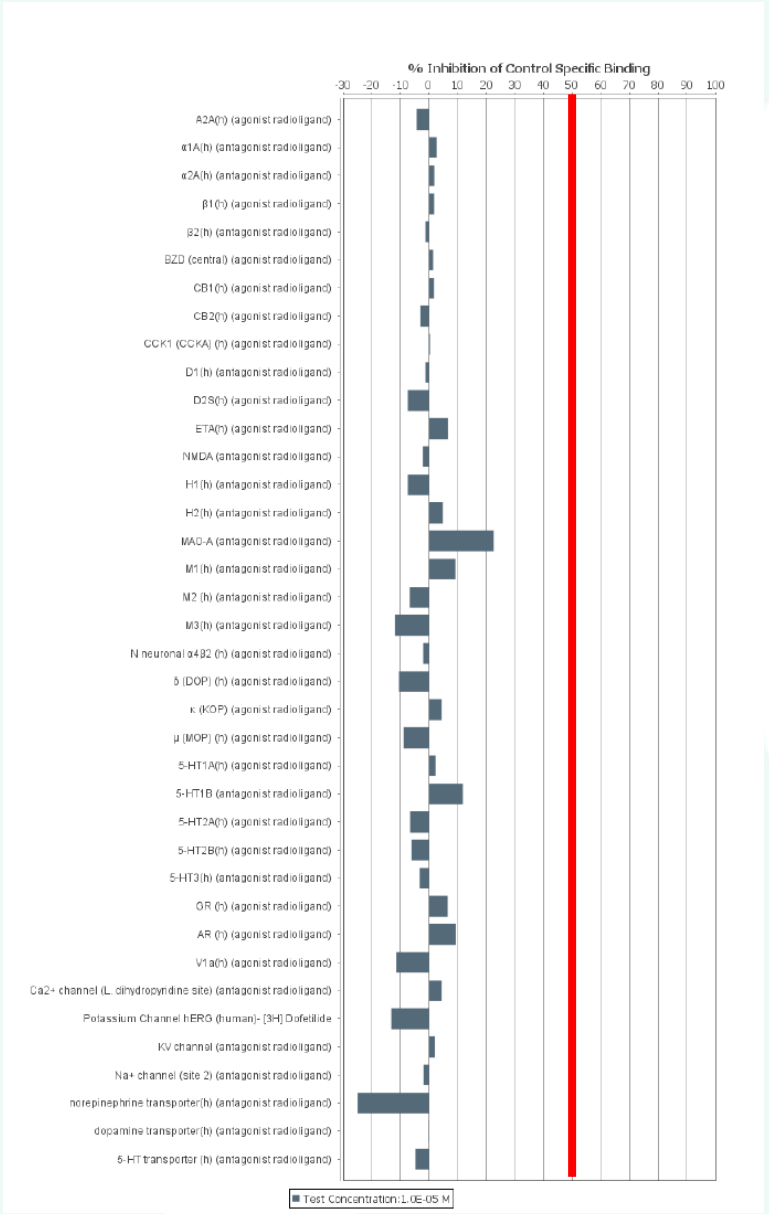
Table 2. Internal Exposures From Use of 0.1% Coumarin in Face Cream and Body Lotion Following the Exposure Scenario Outlined in Table 1

Total Plasma C_{max} (µM)	Mean	Median	90th Percentile	95th Percentile	97.5th Percentile	99th Percentile
Body lotion	0.01	0.01	0.018	0.019	0.02	0.022
Face cream	0.0022	0.0021	0.004	0.0043	0.0046	0.005

The key elements in our NGRA approach- example with the coumarin case study



- **To investigate possible interactions between coumarin and the 44 key targets involved in drug attrition**
- **All binding and enzymatic assay results were negative at 10 µM**



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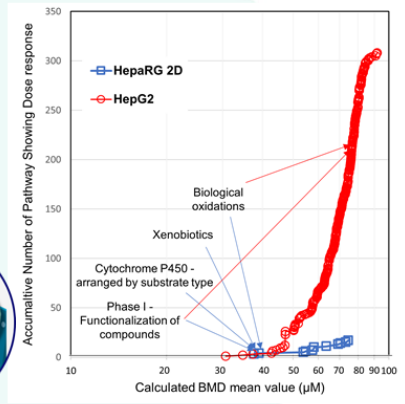
The key elements in our NGRA approach- example with the coumarin case study

Transcriptomics

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

BMDexpress 2



- Transcriptomics was applied as a broad nontargeted biological screen
- Across the cell lines, treatment with coumarin resulted in limited gene-expression changes at concentrations below 100 μM (DESeq2 analysis)
- The MCF7 PoDT were not considered to be sufficiently robust to derive a MoS
- The lowest PoDT for each cell model was selected for the MoS calculation

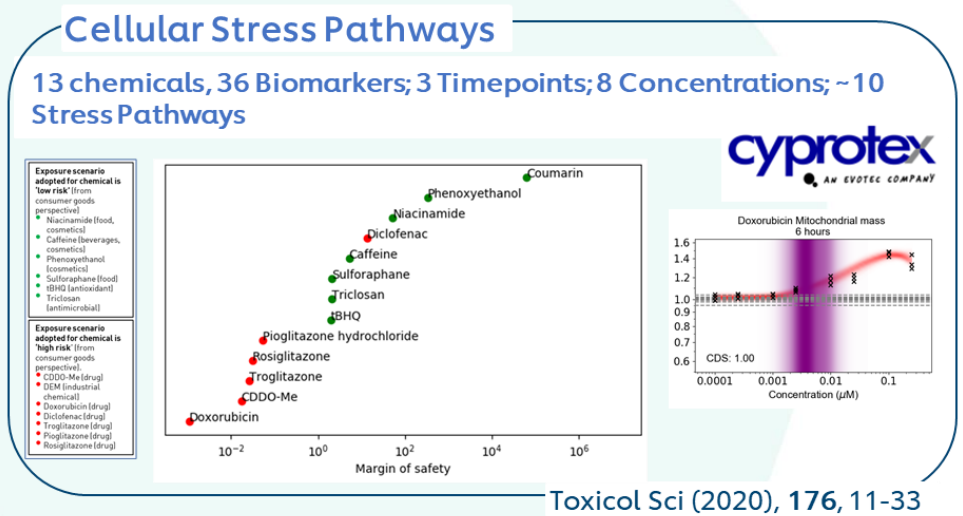
Table 5. PoD_T Values (μM) for Coumarin Treated Across 4 Cell Models for 24 h Using a Subset of Proposed Approaches for Gene Selection Based on Those Proposed by Farmahin *et al.* (2017)

Cell Model	HepG2	MCF7	HepaRG 2D	HepaRG 3D
Pathway-level tests PoD_T (μM)	(308 pathways)	(0 pathways)	(17 pathways)	(2 pathways)
20 pathways with the lowest *p* value Reactome	70	NA	58*	46*
20 pathways with the lowest BMD Reactome	44	NA	58*	46*
BMD of Reactome pathway with lowest BMD that meets significance threshold criteria	31	NA	38	41
Gene-level tests PoD_T (μM)	(1570 genes)	(47 genes)	(87 genes)	(9 genes)
Mean BMD of 20 genes with largest fold change	6	3	54	55
Mean BMD of genes between 25th and 75th percentile	17	1	59	46*

Highlighted (*) are values where the number of pathways or genes was below the recommended number (ie, 20) for grouping. Abbreviation: NA, not applicable.

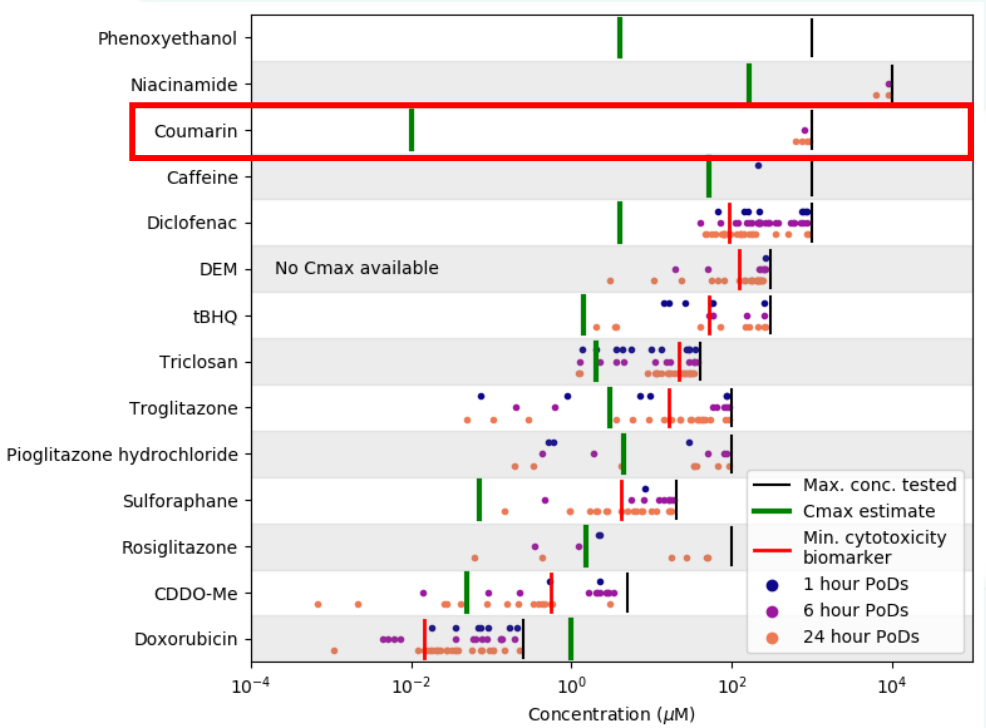
Farmahin, R *et al. Arch Toxicol* **91,** 2045–2065 (2017)

The key elements in our NGRA approach- example with the coumarin case study



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

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



- Bayesian model to quantify the evidence for a biological response – concentration dependency score (CDS) and derive a credibility range for the estimated PoD
- Coumarin not very active in comparison to known “high risk compounds” like doxorubicin
- PoDs shown for HepG2 only

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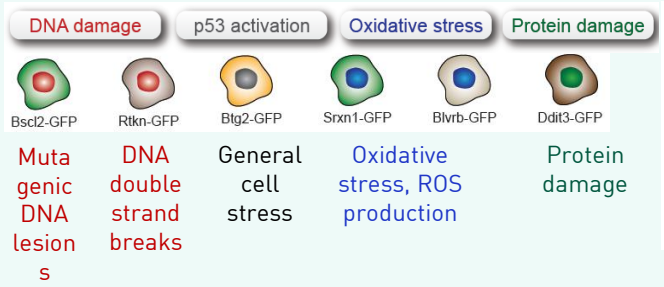
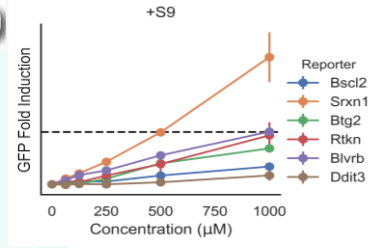
NGRA is hypothesis-driven – examples of bespoke assays used in the coumarin case study

Genotoxicity assessment: ToxTracker®

- **Coumarin and its metabolites triggered genotoxicity alerts**

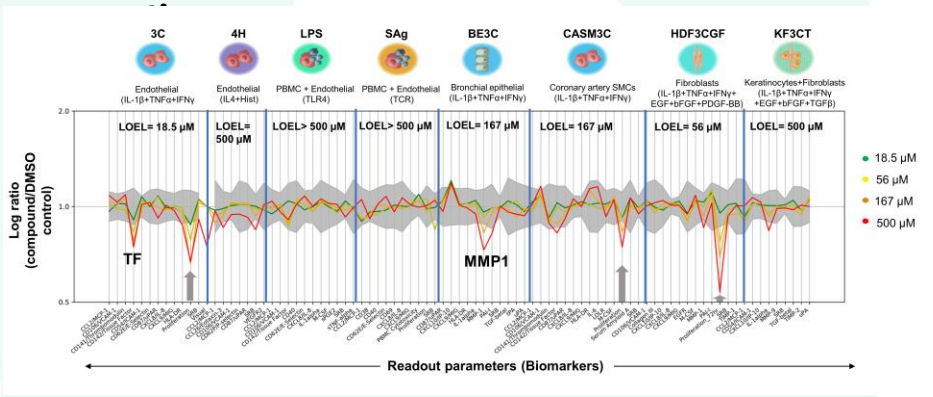




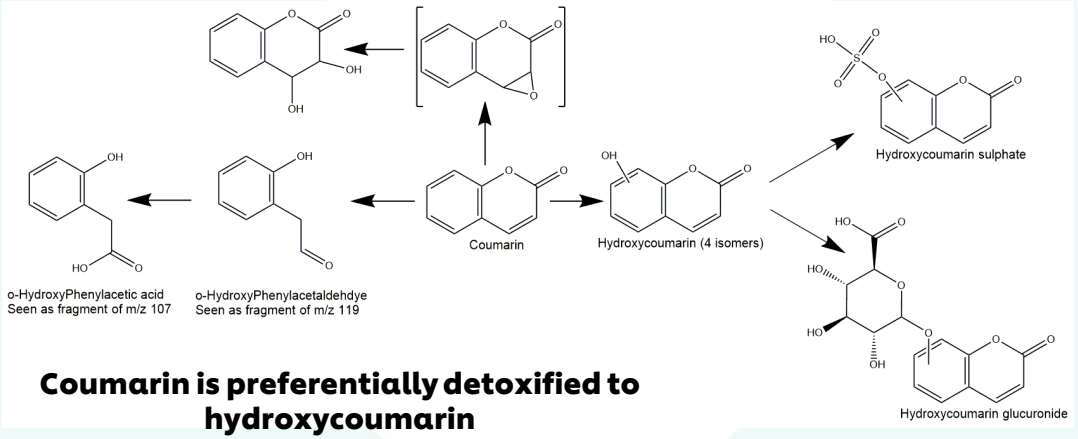
6 GFP reporter mouse embryonic stem (mES) cells

Immunomodulatory screening assay: BioMap® Diversity 8 Panel

- **Coumarin predicted to have anti-inflammatory**



Metabolite identification & PoD refinement

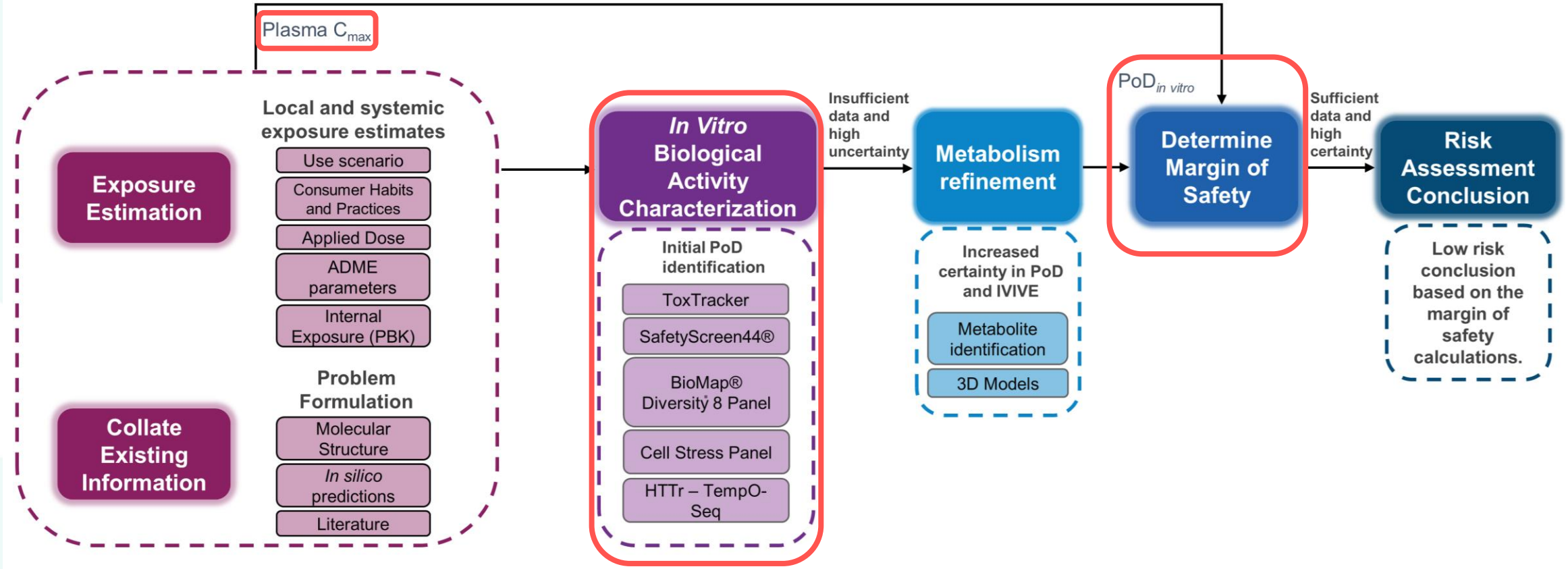


Coumarin is preferentially detoxified to hydroxycoumarin

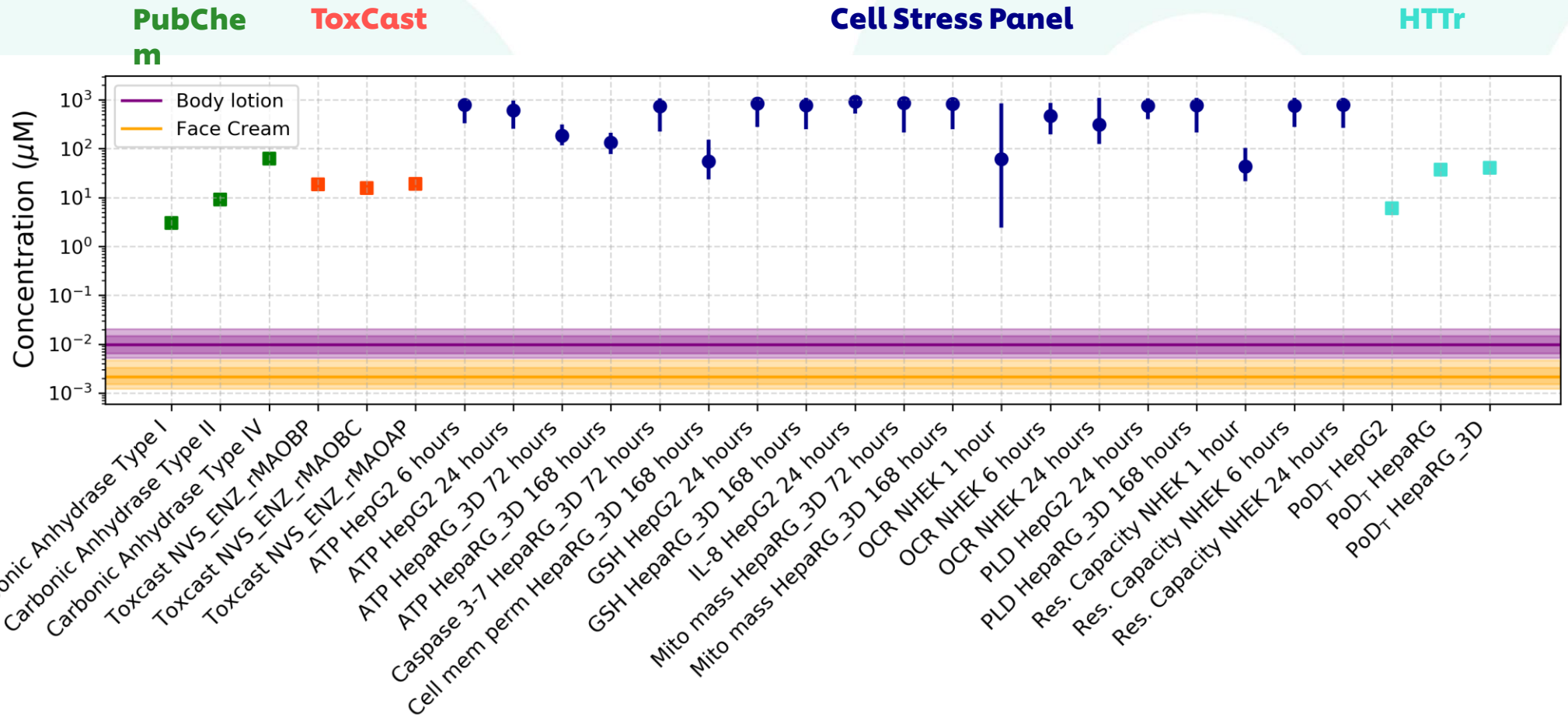
Cell stress & HTTr in 3D HepaRG models

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

Next-Generation Risk Assessment case study workflow



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



- **Coumarin is not genotoxic, does not cause skin sensitisation, does not bind to any of the 44 targets and does not show any immunomodulatory effects at consumer relevant exposures**
- **The 5th percentile of the BER distribution ranged between 158 and 96738**

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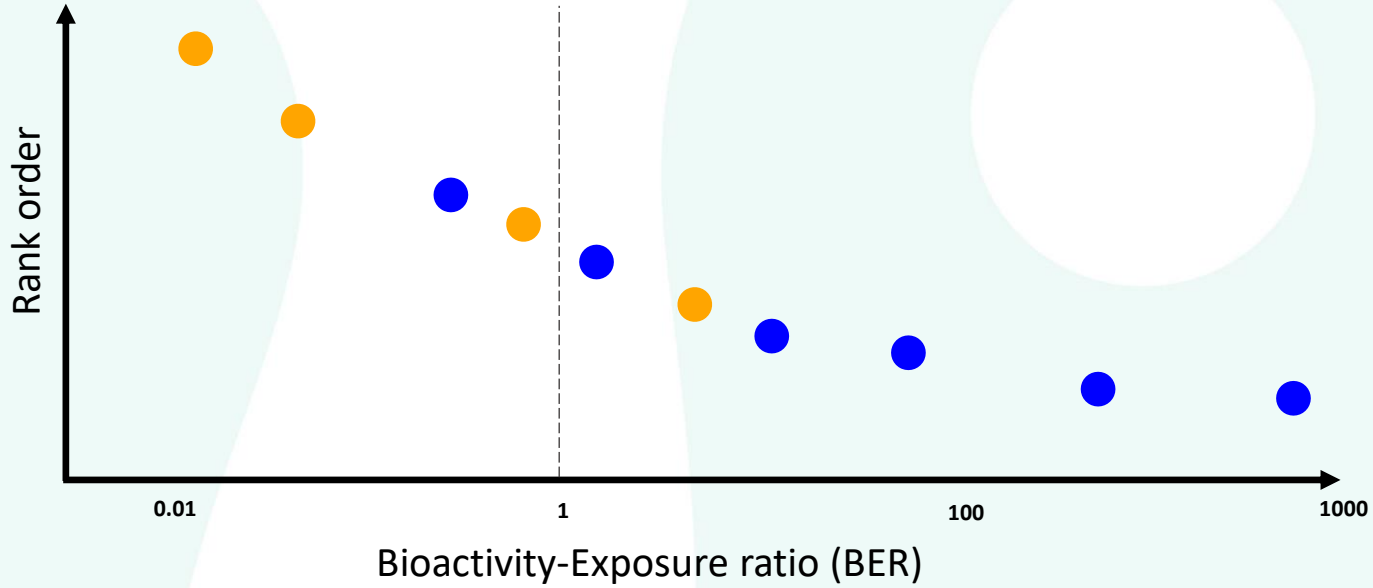
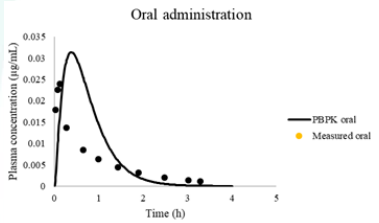
Ongoing work: Evaluating the toolbox for risk assessment- A data driven approach

Chemical exposures scenarios

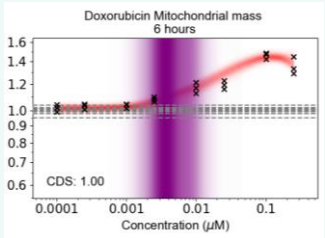
- ‘Low’ risk (from consumer goods perspective) – e.g. foods, cosmetics
- ‘High’ risk (from consumer goods perspective) – e.g. drugs



Define typical use-case scenarios benchmark chemical-exposures



PBK models of systemic exposure



Calculate the PoDs

Calculate the Bioactivity-Exposure ratio

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

Concluding remarks

- NAMs can provide robust insights to support exposure estimation and mechanistic in vitro bioactivity data to inform non-animal safety assessment- data generation is driven by the risk assessment questions
- The approach focuses on building a weight of evidence- tools can be integrated to make a safety decision
- NGRA aims to be protective of human health at defined exposures- consideration of both bioactivity and levels of exposure
- Evaluation of NGRA needs to be in the context of how to combine (often many different) estimates of exposure and bioactivity give reproducible decisions on safety with transparent measurement of uncertainty
- For validation of this approach there is a need for:
    - Well curated chemical/exposure scenarios that have documented history of safety/ non-safety in humans or chemical/exposure scenarios recognised from historical risk assessments as being safe/non-safe
- There is a need to increase confidence amongst many risk assessors with the use of mathematical approaches in NGRA used to combined different types of in vitro data (PBK modelling, PoD modelling etc)
- A proactive evaluation of MoS derived with NGRA for defined chemical/exposure scenarios will add to the growing information on the degree of protection provided by risk assessments based on human exposure and biology rather than on trying to predict high dose effects in animal
- Through the process of this evaluation we can identify gaps in our approaches and design new testing strategies to address them. E.g. where can more advanced tools such as microphysiological systems be useful in NGRA?

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Acknowledgements

Core Team:

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