

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

Maria Baltazar



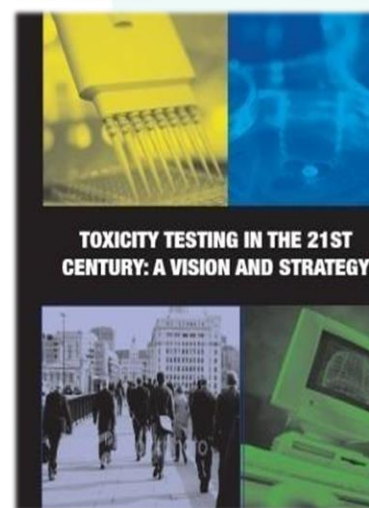
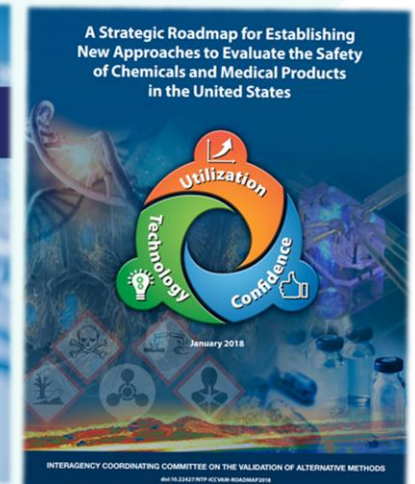
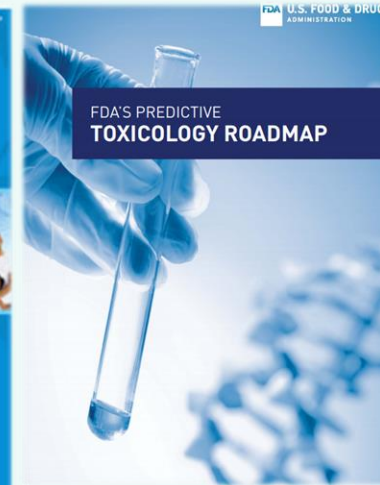
Unilever

Next Generation Risk Assessment (NGRA)

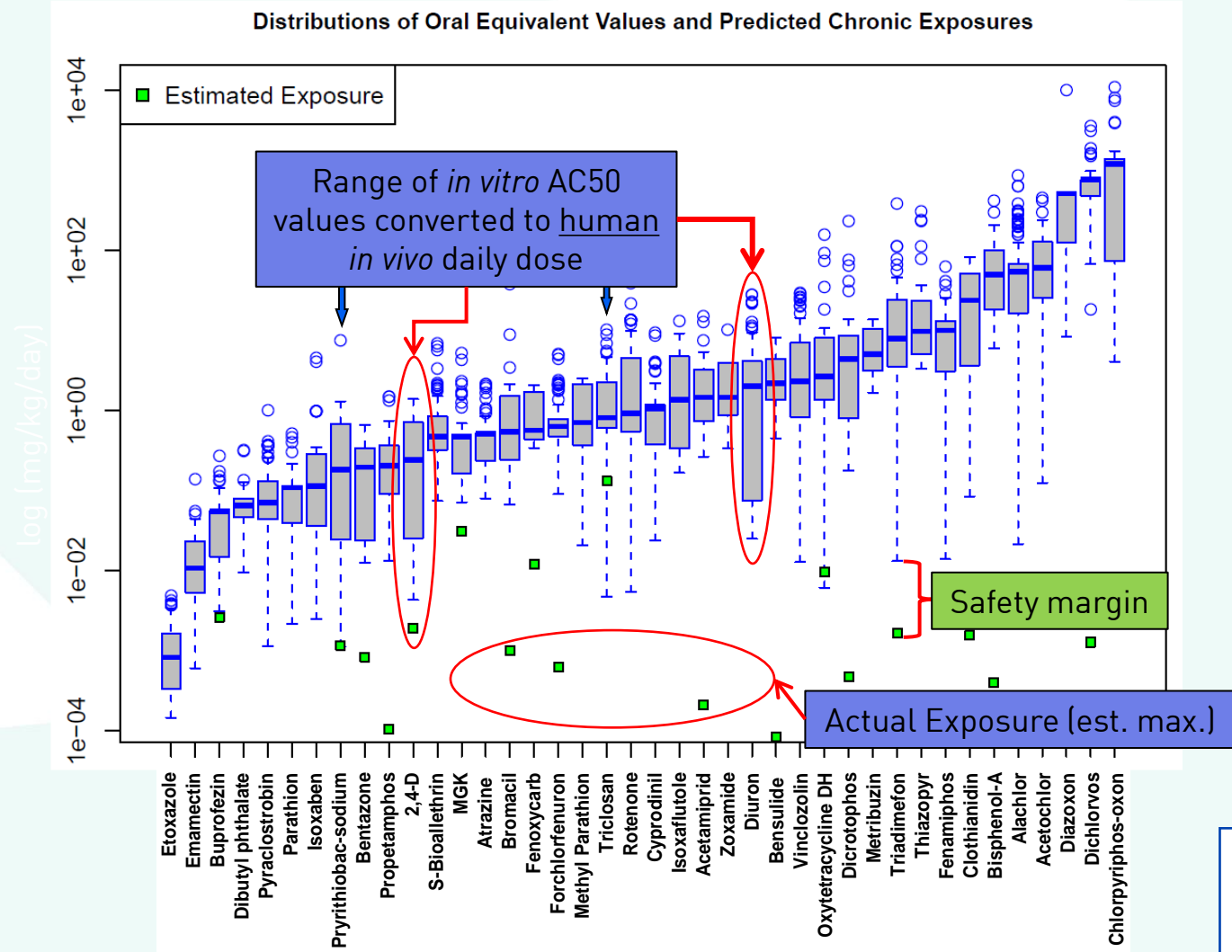
NGRA is defined as ***an exposure-led, hypothesis-driven*** risk assessment approach that ***integrates New Approach Methodologies (NAMs)*** to assure ***safety without the use of animal testing***



Safety without animal testing



A fundamental principle of NGRA: '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.**

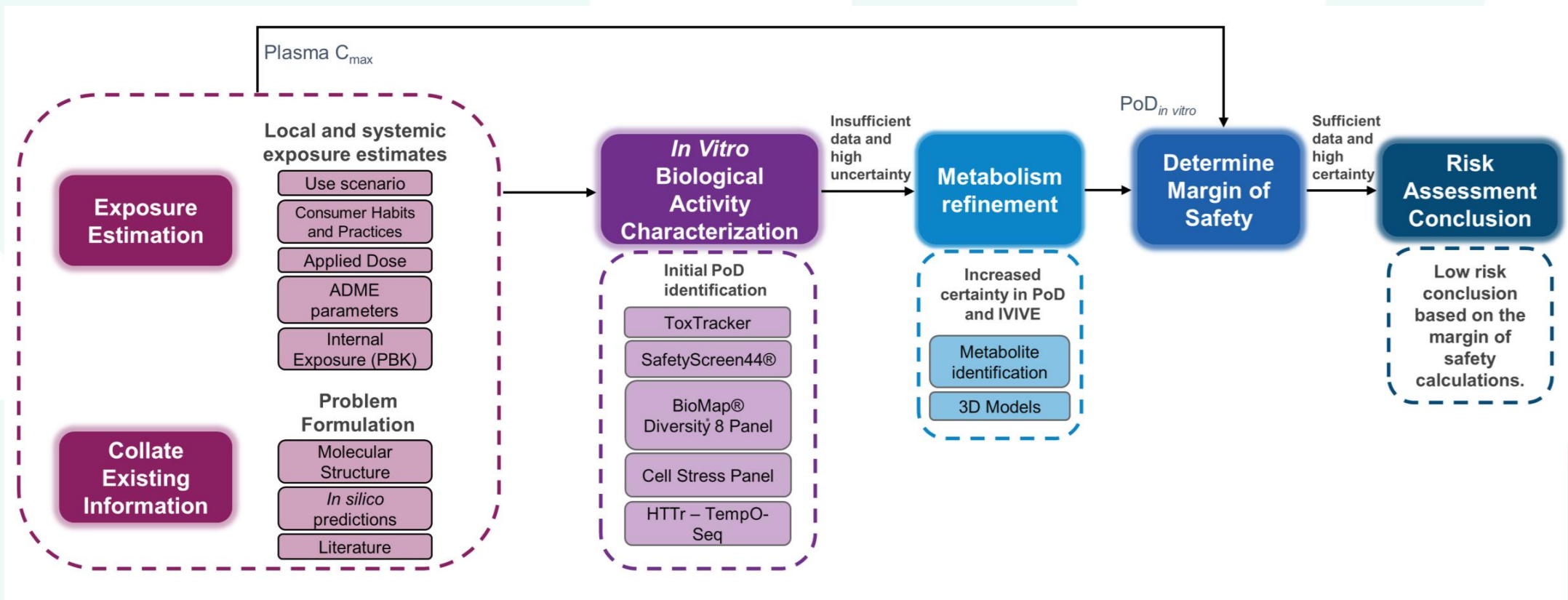
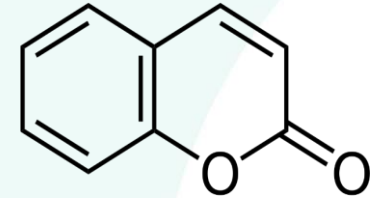
Slide from Dr Rusty Thomas, EPA, with thanks

Rotroff, et al. Tox.Sci 2010



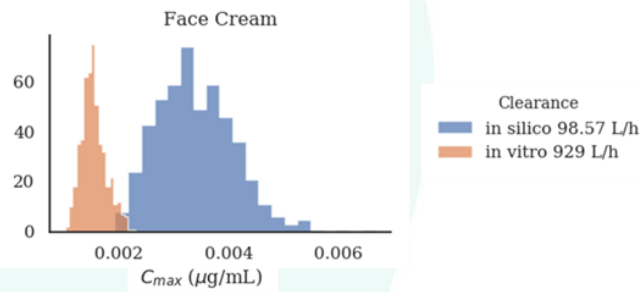
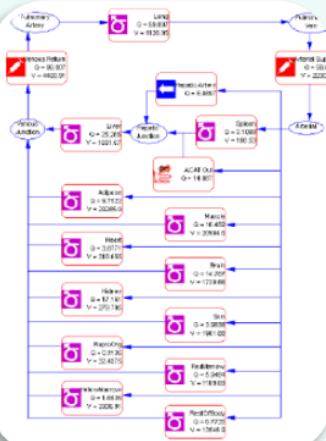
A case study approach – human health safety assessment required for...

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



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

Joanne Brown, Andrew J. Brown, Jacques Héman, Wolfgang Jorntink, Arun Sridhar, Gareth Waldron and Steven Whitbread

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.

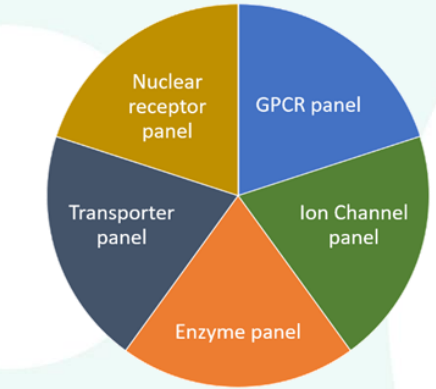
Decreasing the high attrition rate in the drug discovery and development process is a primary goal of the pharmaceutical industry. One of the main challenges in achieving this goal is striking an appropriate balance between drug efficacy and potential adverse effects as early as possible in order to 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, being in mind the increasing demand and regulatory constraints target (or targets), whose 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 *in vitro* pharmacology assay that is absolutely required by regulatory authorities is one that measures the effects of new chemical entities on the ionotropic calcium (Ca_v) or heteromultimeric expressed human voltage-gated potassium channel subfamily II member 2 (hKCNH2), also known as hERG. 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^{1,2}, 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 entities³.

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 need for most pharmaceutical companies 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.

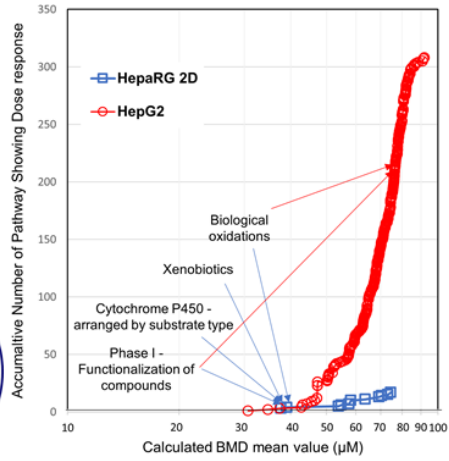
Here, for the first time, four major pharmaceutical companies (AstraZeneca, GlaxoSmithKline, Novartis and Pfizer) share their best knowledge and experience of the innovative application of existing screening technologies to detect off-target interactions of compounds. The objective of this article is to describe the rationale and main advantages for the use of *in vitro* pharmacological profiling to discuss best practices and to



Transcriptomics

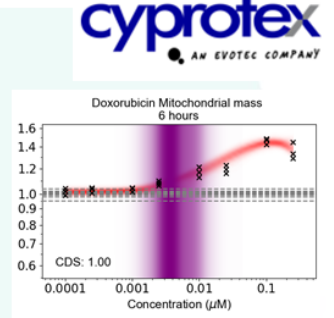
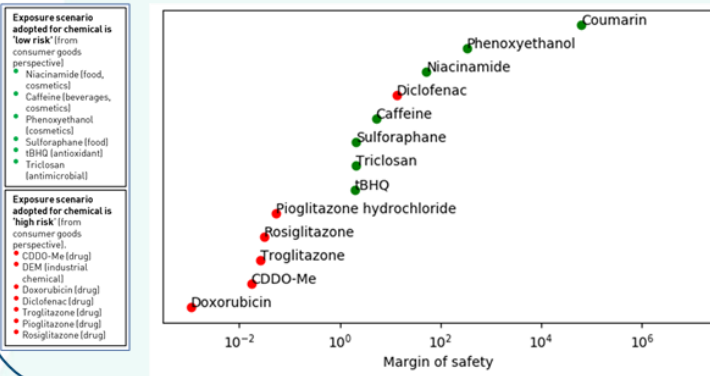
- Use of full human gene panel ~ 21k
- 24 hrs exposure
- 7 concentrations
- 3 cell lines HepG2/ HepaRG/ MCF7
- 3D HepaRG spheroid

BMDexpress 2



Cellular Stress Pathways

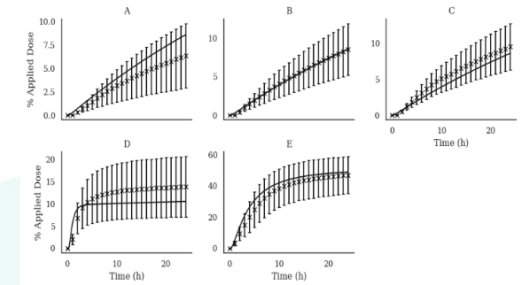
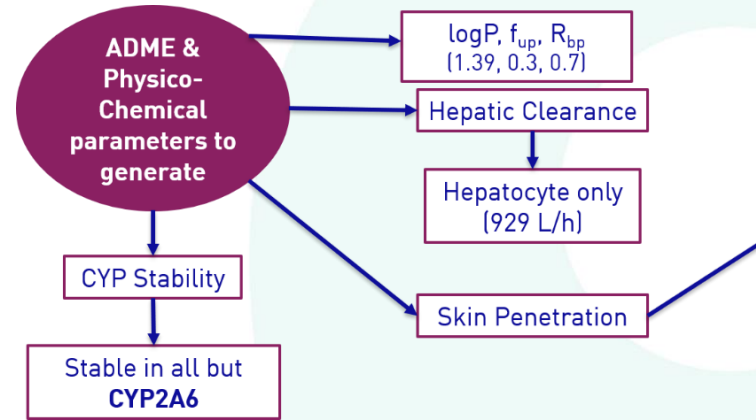
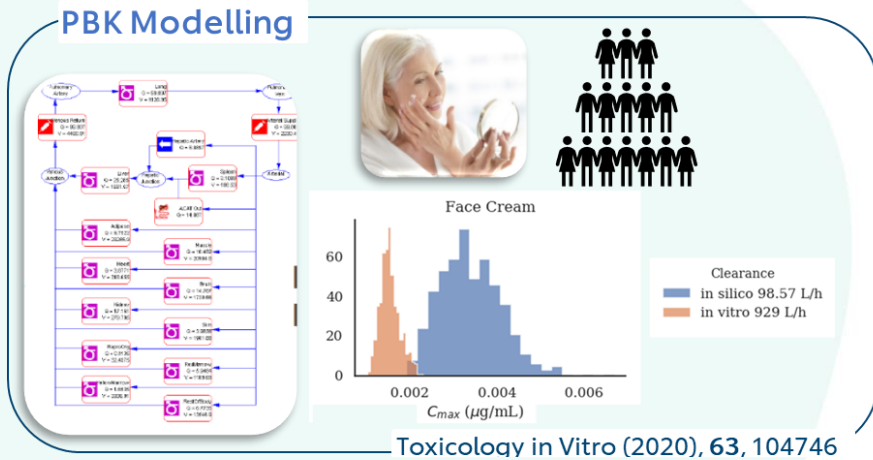
13 chemicals, 36 Biomarkers; 3 Timepoints; 8 Concentrations; ~10 Stress Pathways



Toxicol Sci (2020), 176, 11-33



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



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}

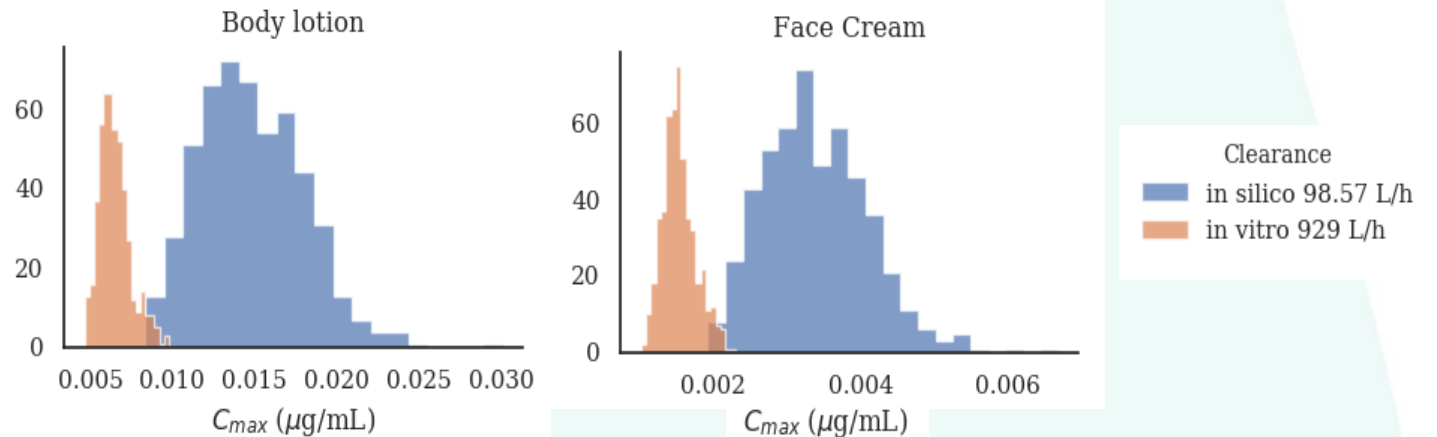


Table 2. Internal Exposures From Use of 0.1% Coumarin in Face Cream and Body Lotion Following the Exposure Scenario Outlined in Table 1

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

In vitro pharmacological profiling

PERSPECTIVES

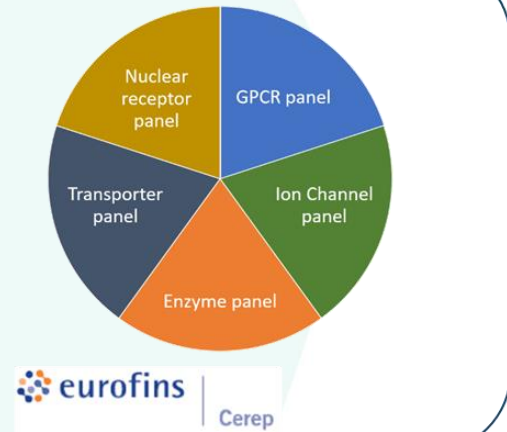
REDUCING SAFETY-RELATED DRUG ATTRITION: THE USE OF *IN VITRO* PHARMACOLOGICAL PROFILING

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 (Amgen, 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.

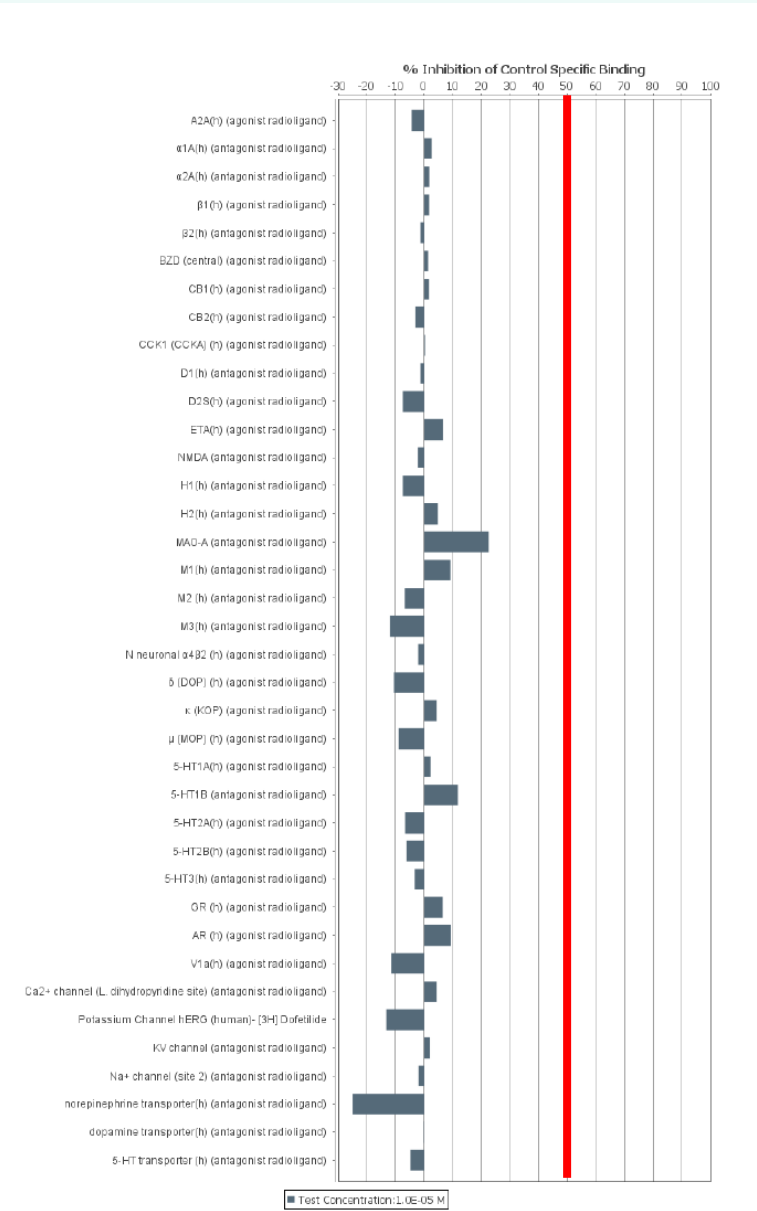
Decreasing the high attrition rate in the drug discovery and development process is a primary goal of the pharmaceutical industry. One of the main challenges in achieving this goal is writing an appropriate balance between drug efficacy and potential adverse effects as early as possible in order to reduce safety-related attrition, particularly in the more expensive late stages of clinical development. Gaining better understanding of the safety profile of drug candidates early in the process is also crucial to reducing the likelihood of safety issues leading to the approval of drugs or even leading to their market withdrawal, having in mind that the cost of drug development is increasing exponentially.

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 pharmacological 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 target receptors, ion channels, enzymes and transporters that are known from the literature,



- **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**

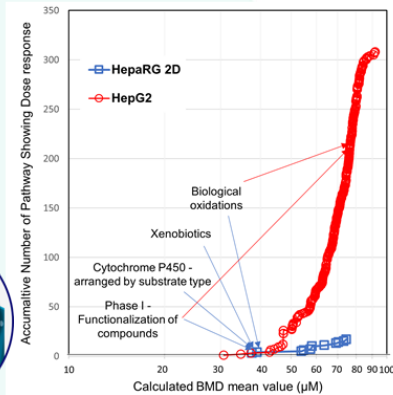
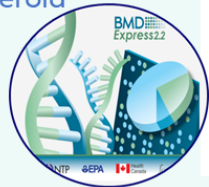


The key elements in our NGRA approach- example with the coumarin case study

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. \(2017\)](#)

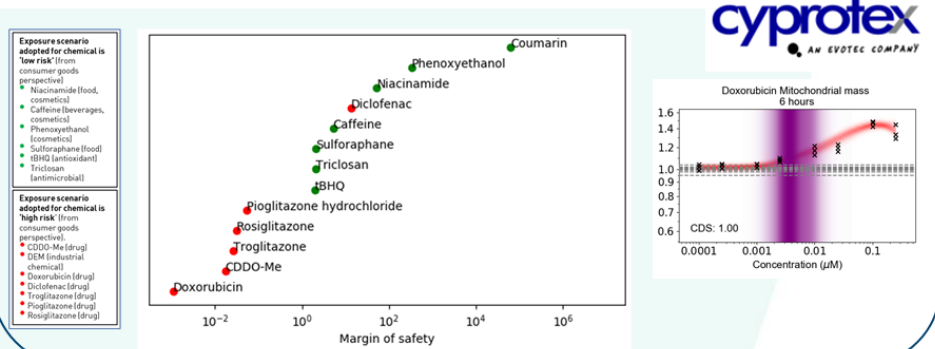
Cell Model	HepG2	MCF7	HepaRG 2D	HepaRG 3D
Pathway-level tests PoD _T (µM)	(308 pathways)	(0 pathways)	(17 pathways)	(2 pathways)
20 pathways with the lowest p value Reactome	70	NA	58*	46*
20 pathways with the lowest BMD Reactome	44	NA	58*	46*
BMD of Reactome pathway with lowest BMD that meets significance 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.

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

Cellular Stress Pathways

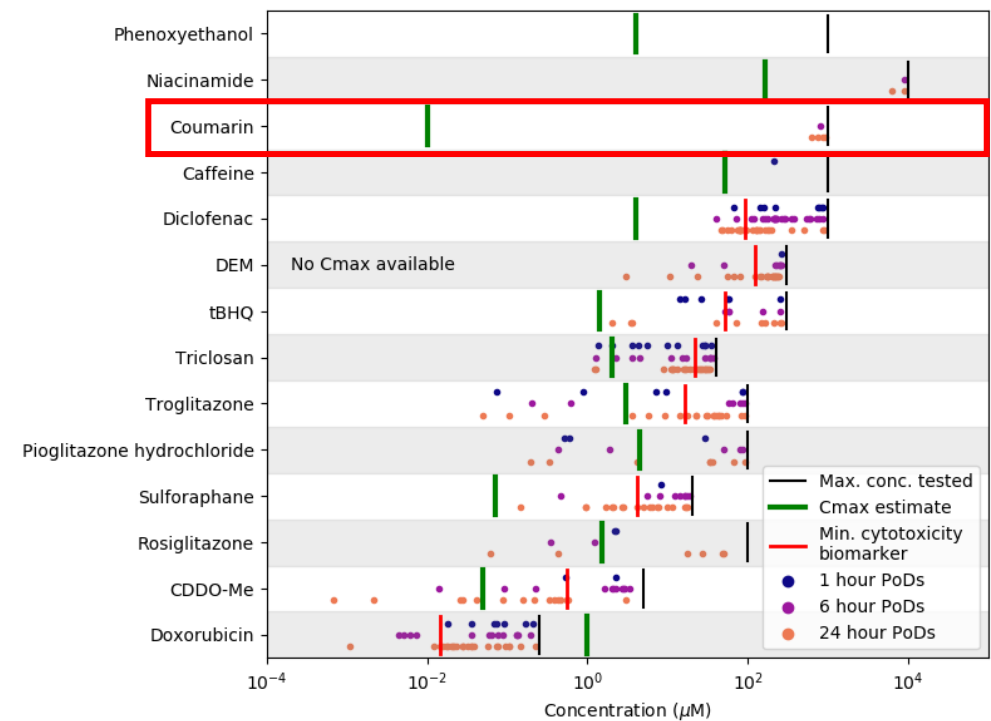
13 chemicals, 36 Biomarkers; 3 Timepoints; 8 Concentrations; ~ 10 Stress Pathways



Toxicol Sci (2020), 176, 11-33

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

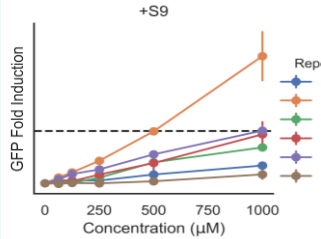
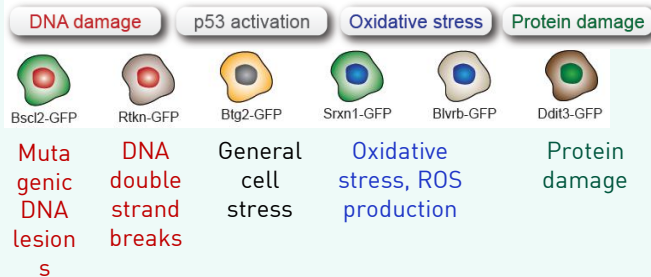
- Bayesian model to quantify the evidence for a biological response – concentration dependency score (CDS) and derive a credibility range for the estimated PoD
- Coumarin not very active in comparison to known “high risk compounds” like doxorubicin
- PoDs shown for HepG2 only



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

Genotoxicity assessment: ToxTracker®

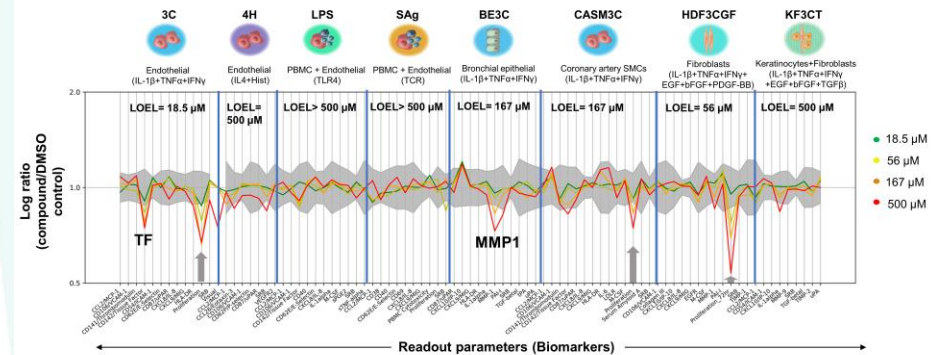
- Coumarin and its metabolites triggered genotoxicity alerts



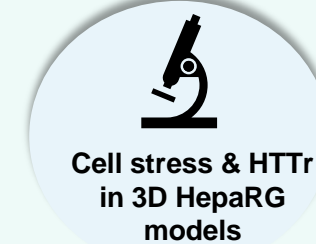
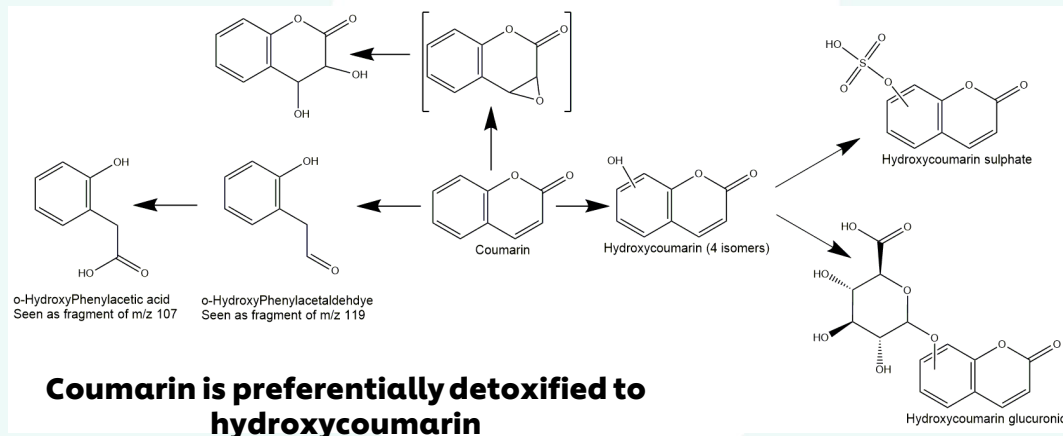
6 GFP reporter mouse embryonic stem (mES) cells

Immunomodulatory screening assay: BioMap® Diversity 8 Panel

- Coumarin predicted to have anti-inflammatory

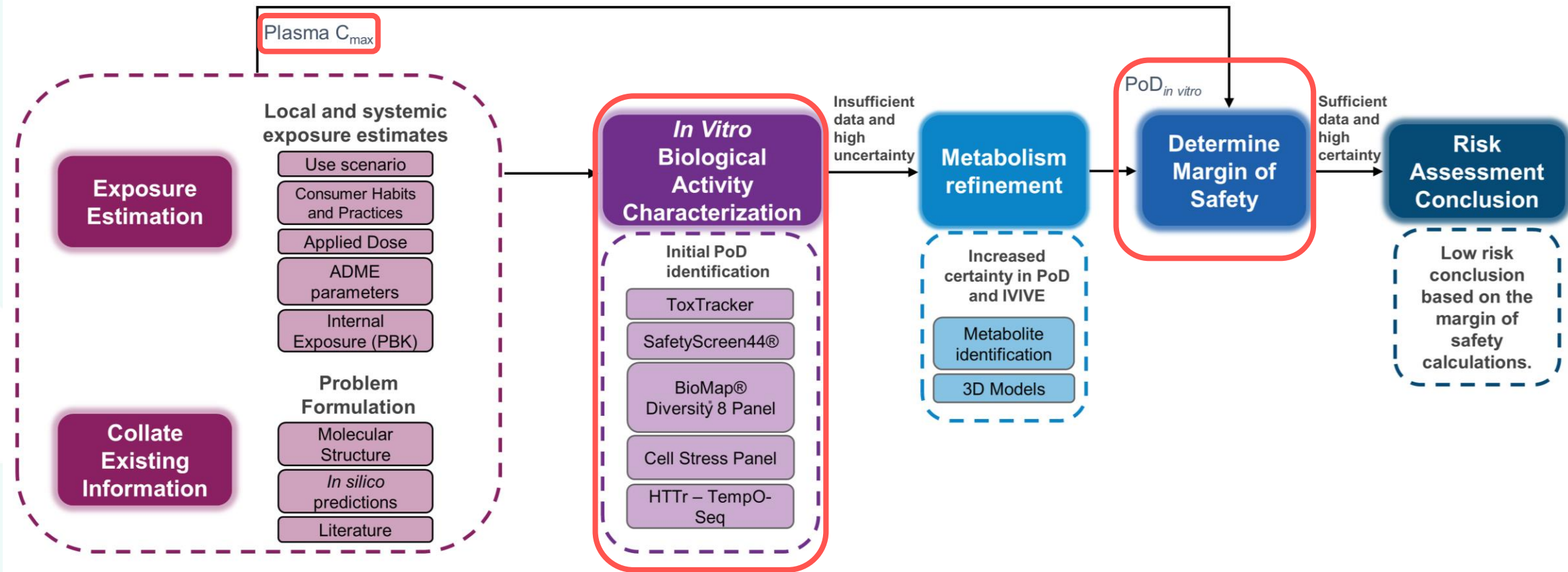


Metabolite identification & PoD refinement

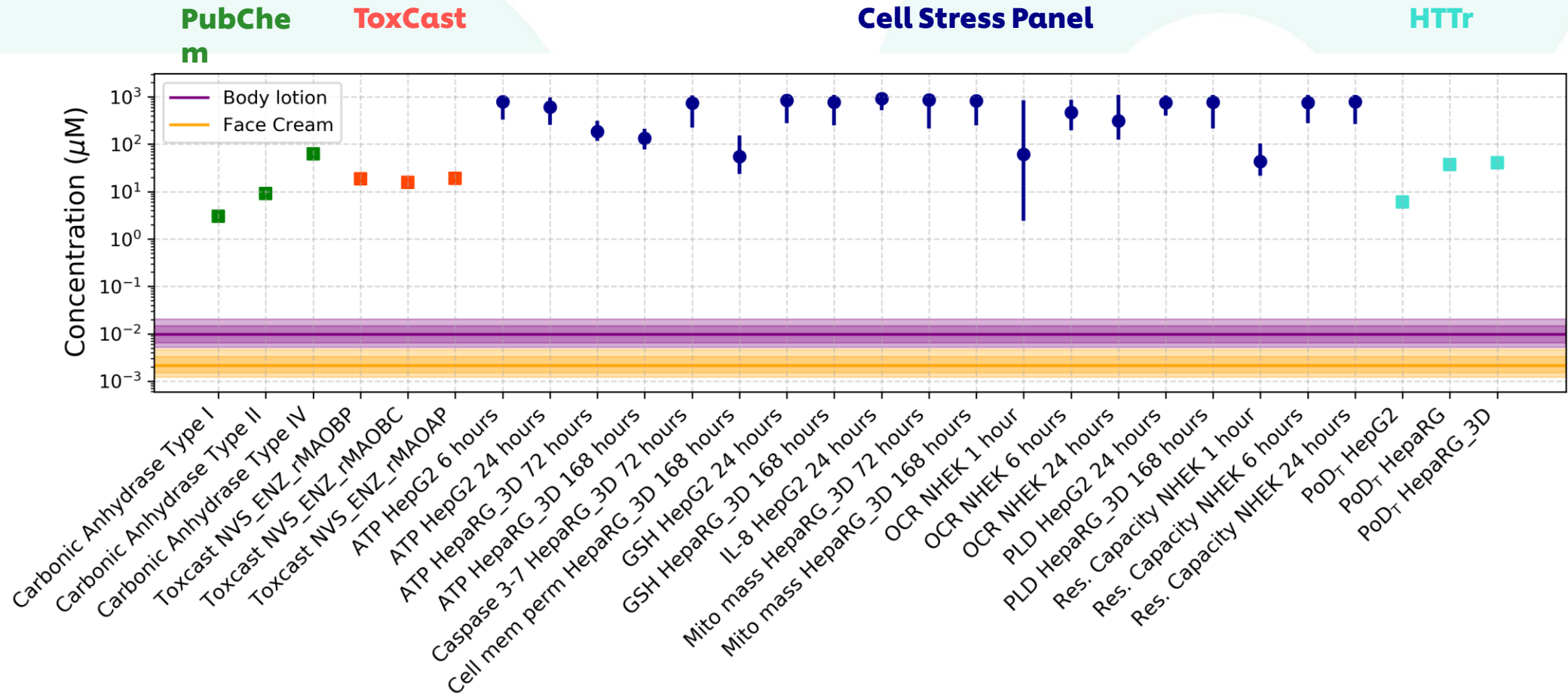


- Low bioactivity also found in a metabolic competent cell model (HepaRG 3D)
- PoDs range: 41-871 µM – similar range as in from 2D cells

Next-Generation Risk Assessment case study workflow



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

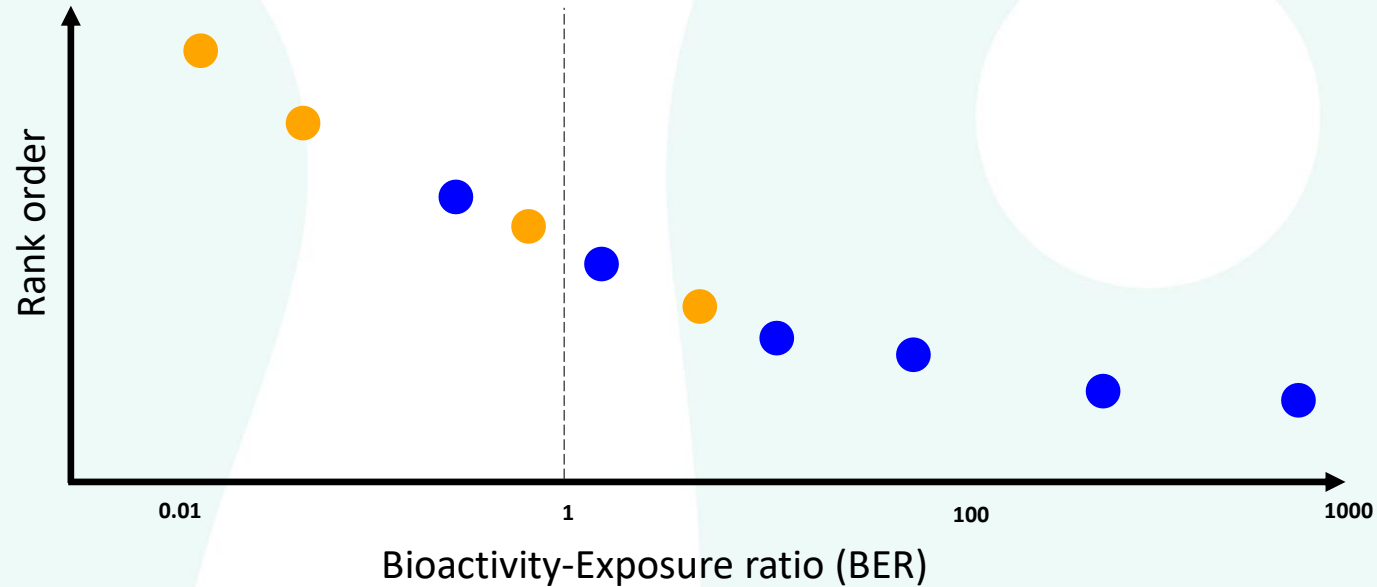


- **Coumarin is not genotoxic, does not cause skin sensitisation, does not bind to any of the 44 targets and does not show any immunomodulatory effects at consumer relevant exposures**
- **The 5th percentile of the BER distribution ranged between 158 and 96738**

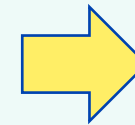
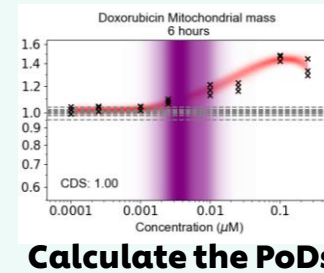
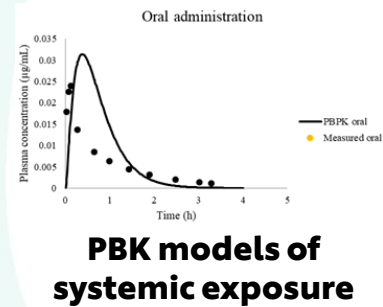
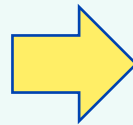
Ongoing work: Evaluating the toolbox for risk assessment- A data driven approach

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



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?

Acknowledgements



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- Carl Westmoreland, Paul Russell, Gavin Maxwell, Ian Sorrell, Sam Piechota, Juliette Pickles, Karen Bonner, Sandrine Spriggs, Iris Muller, Katarzyna Przybylak, Paul Walker, Caroline Bauch, Rebecca Beaumont, Steve Clifton, Katie Paul-Friedman, Julia Fentem