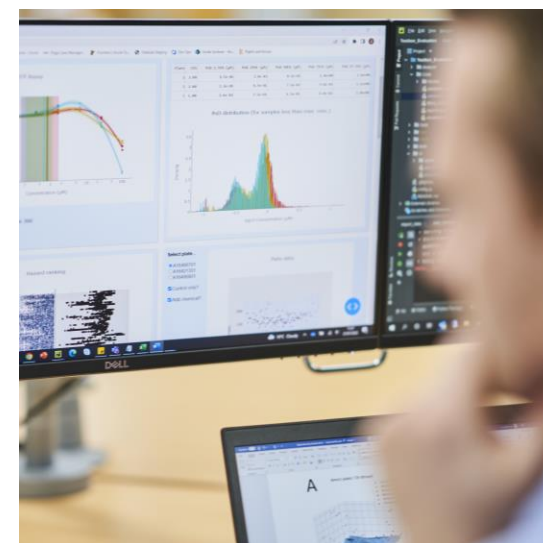
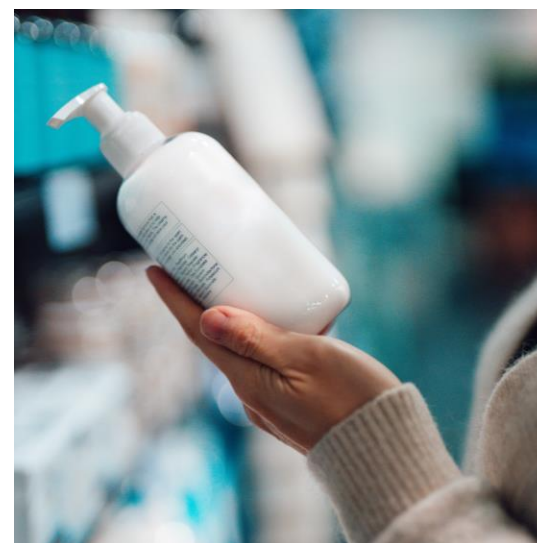


Next Generation Risk Assessment: An industry perspective on the application of toxicogenomics and the challenges for implementation

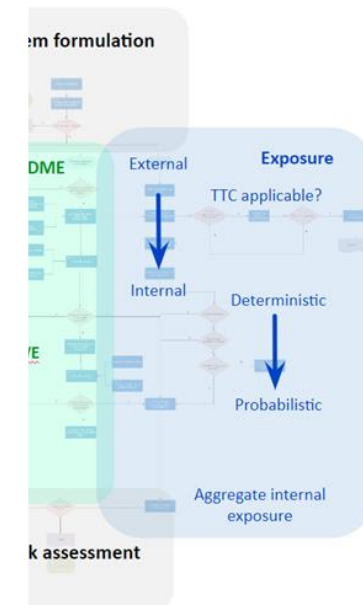
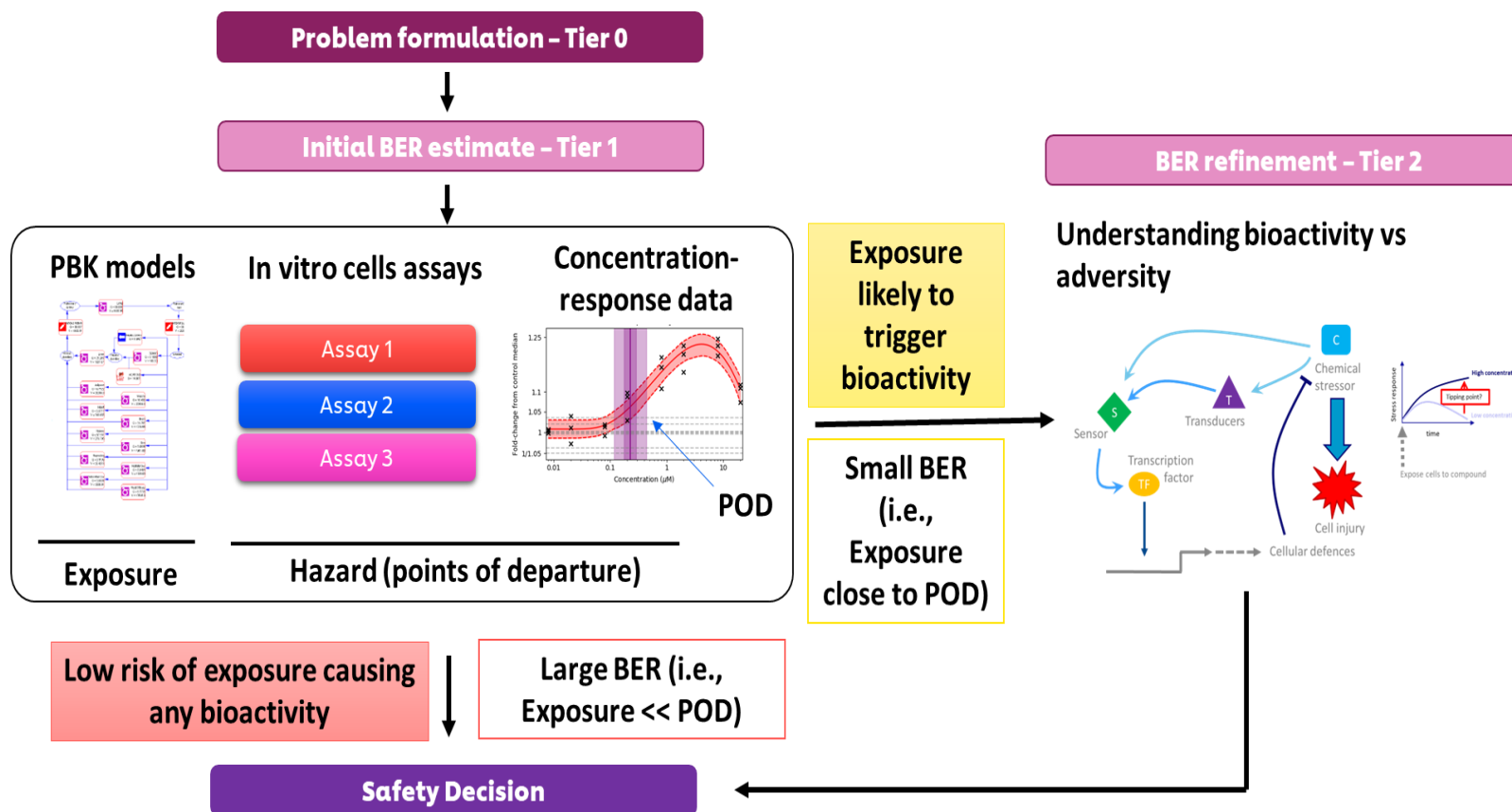
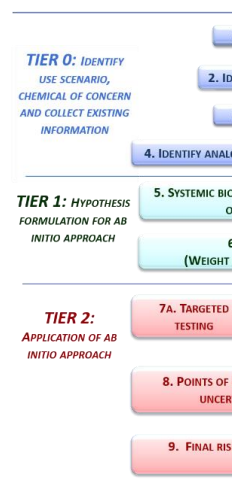
Dr Andrew White
Unilever Safety and Environmental Assurance Centre

Session 24: Toxicogenomics: breaking barriers for regulatory implementation

13th September 2023



Decision frameworks in NGRA



Objective Application of Omics for NGRA



- Determination of Compounds primary MoA through pathway analysis
- Underpinning AOP key events and MIEs

- Comparison of differentially expressed signatures from one treatment to a database of previously reported gene signatures or to another sample.

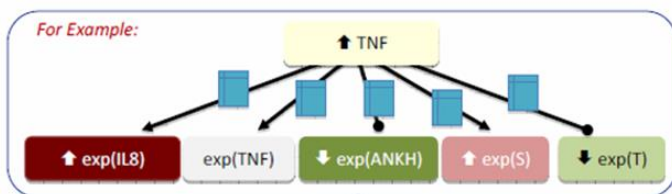
- Dose response relationship and identification of biologically relevant dose

Prediction

Protection

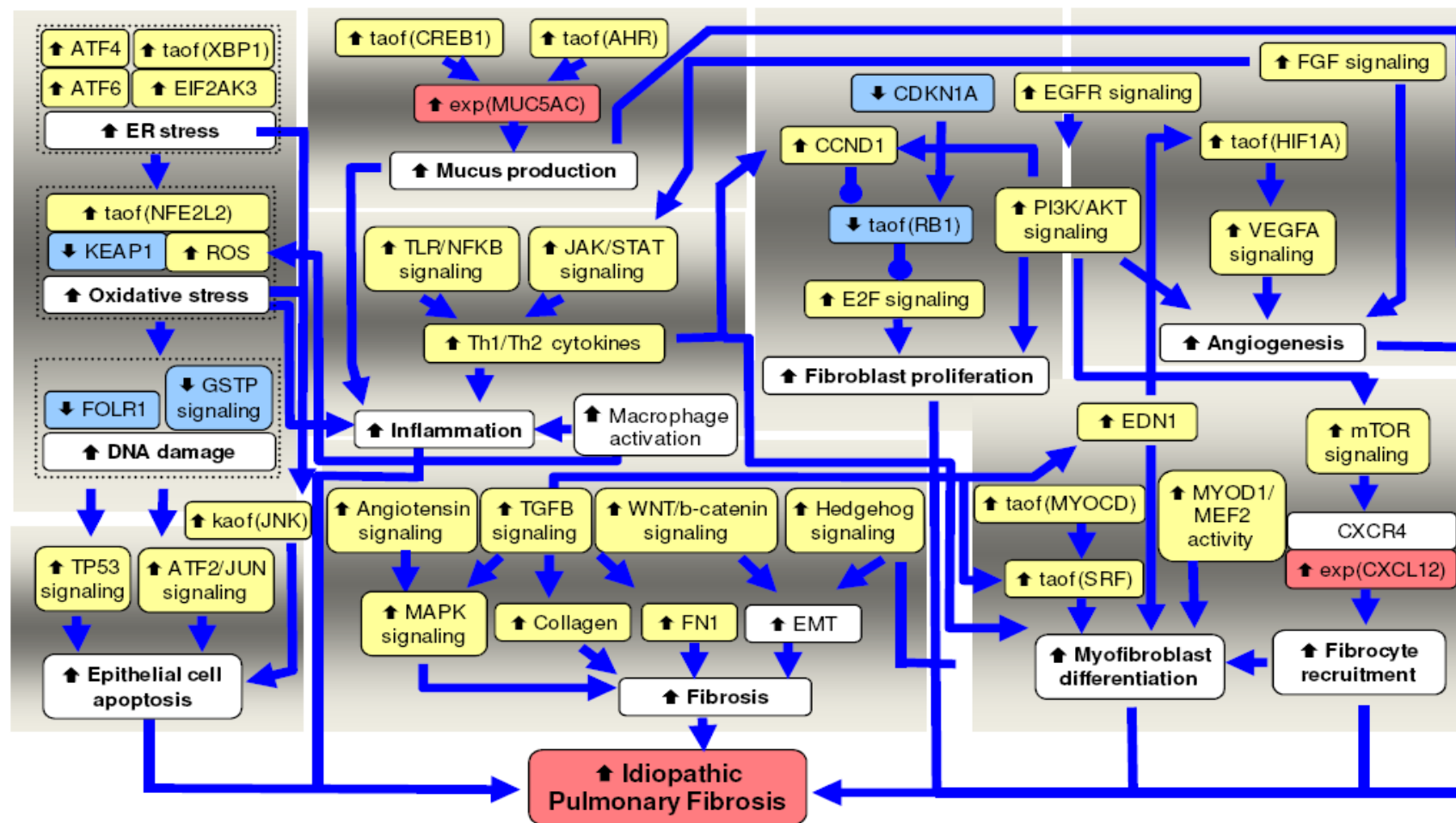
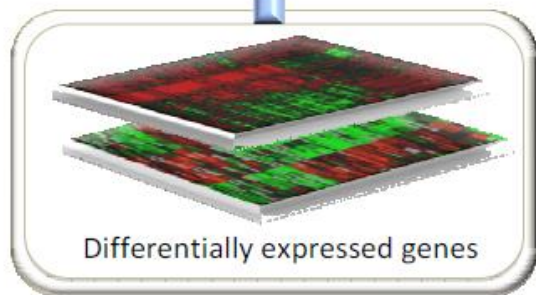
However : acceptance of omics data to support the hazard/safety assessment is still limited. Due to a combination of complexity, rapid developments, no defined ground truth

Developing a Data driven AOP



Identification of mechanistic causes leading to differential gene expression changes

Reverse Causal Reasoning

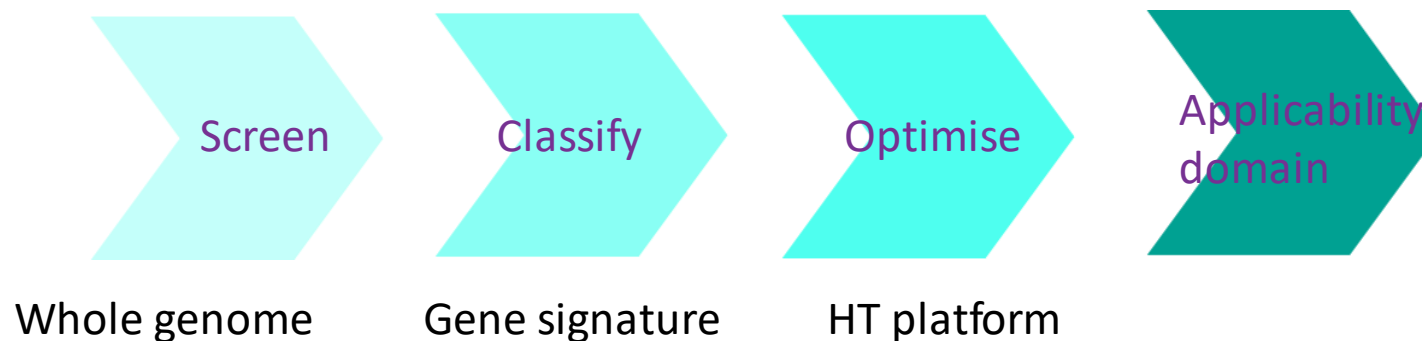


Example Classification approaches – Gene Signature / Gene Signature Database

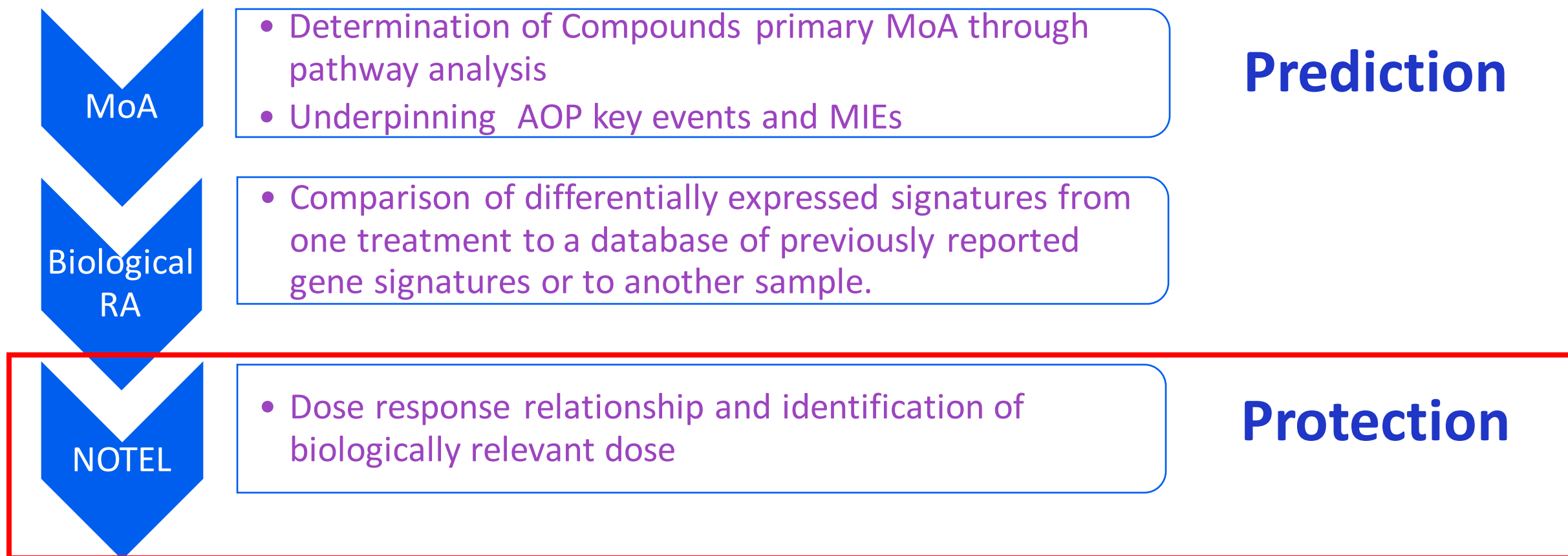
- Still an area of active development especially seeing new developments in use of AI
 - Tends to provide clearer data on more potent compounds due to stronger signal to noise values
 - Signatures for classifiers
- Concerns around transferability across different cell lines
 - Requires approaches that minimises FP rate
 - Platform limitations of comparative Databases
 - Shown to be used for cosmetic relevant ingredients – parabens Naciff et.al 2022

GARD - skin sensitisation and

TGx-DDI - Gentox

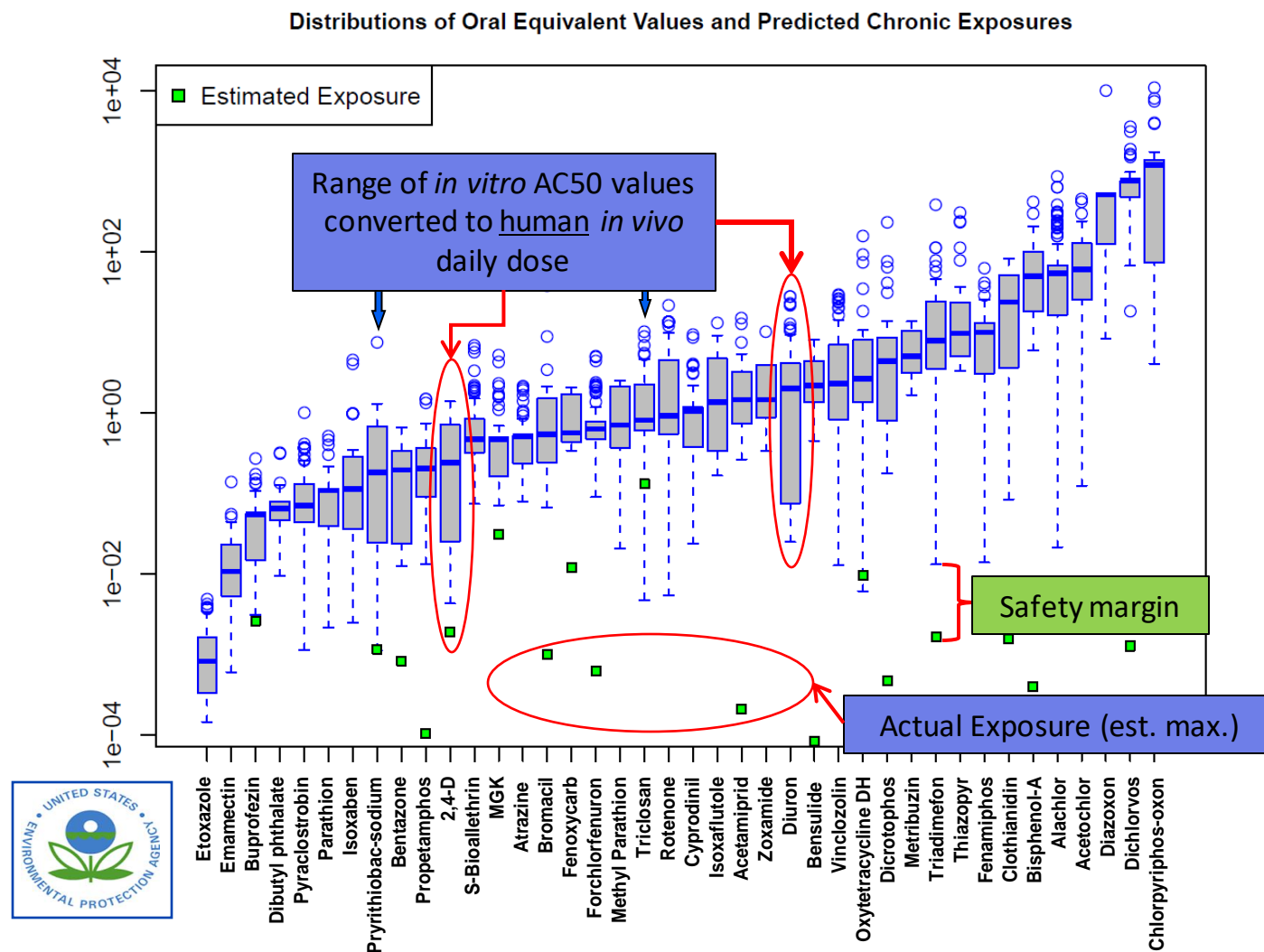


Objective Application of Omics for NGRA



However : acceptance of omics data to support the hazard/safety assessment is still limited. Due to a combination of complexity, rapid developments, no defined ground truth

Paradigm shift for systemic safety - 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.

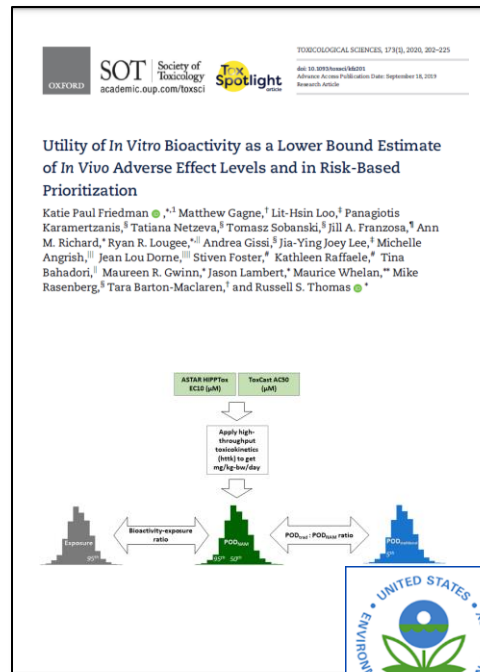
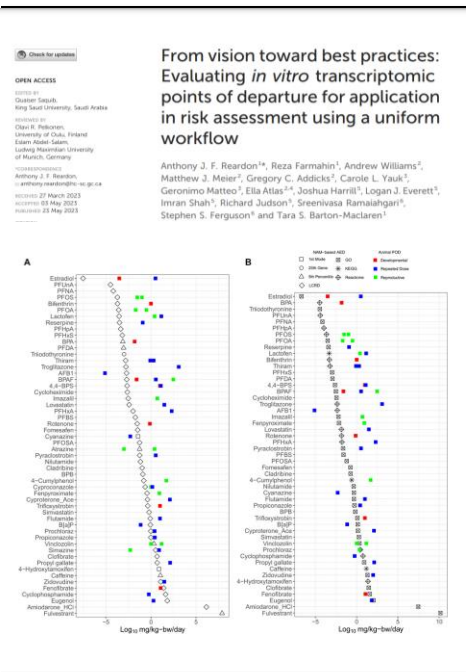
Aligns to an exposure led framework where estimates of consumer product ingredients can be determined



Slide from Dr Rusty Thomas,
EPA, with thanks

Rotroff, *et al.* Tox.Sci 2010

Examples of ongoing or completed case studies for NAM/NGRA BER based risk assessment or prioritisation



Science Approach Document

Bioactivity Exposure Ratio: Application in Priority Setting and Risk Assessment

Health Canada

March 2021

The flowchart illustrates the process of calculating the Bioactivity Exposure Ratio (BER) and its application in risk assessment. It starts with two input parameters: ASTAR HiPPFes (EC50 μM) and ToxCast AC50 (μM). These are used to calculate the Bioactivity exposure ratio (BER), which is then compared to a threshold (BER_{thr}). If the BER is greater than or equal to the threshold, it leads to the calculation of the PODs/POD_{low} ratio. This ratio is then used to determine the final POD (Point of Departure) for risk assessment.

OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2021)35

Unclassified English - Or. English
27 October 2021

**ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE**

Case Study on use of an Integrated Approach for Testing and Assessment (IATA) for Systemic Toxicity of Phenoxyethanol when included at 1% in a body lotion

Series on Test No. 349

Cosmetics Europe
the personal care association

COSMETICS EUROPE LRSS

EUTOXRISK

An Integrated European 'Flagship' Program
Driving Mechanism-based Toxicity Testing and Risk Assessment for the 21st Century

Case Study 16 Reporting Template

Team: 2

Team Members: Barira Islam; Ugis Sarkans; Marcel Leist Alessandra Roncaglioni; Jukka Sund; Andrew White.

Compound ID: CS_16-02
Compound Name: (4-Hydroxy-2,2,6,6-tetramethylpiperidin-1-yl)iodoacetate; TEMPOL
Structure:

Ab Initio Case Study Objectives

Scientific Objectives

- Establish ability of NAM to establish a point of departure for risk assessment.
- Use NAM to assess the risk of systemic toxicity of phenoxyethanol when included at 1% in a body lotion.
- Validate NAM approaches within the risk assessment context that they can be used for risk assessment.
- Compare the results with the goal of NAM to establish a point of departure for regulatory testing.

People Objectives

- Provide risk assessment experience for partners to develop skills, many of whom are not currently conducting risk assessment that they do the work.
- Develop a network of experts in NAM to support the work.
- Develop a network of experts in NAM to support the work.

Other Identifiers: CAS ID 2226-96-2; CHE

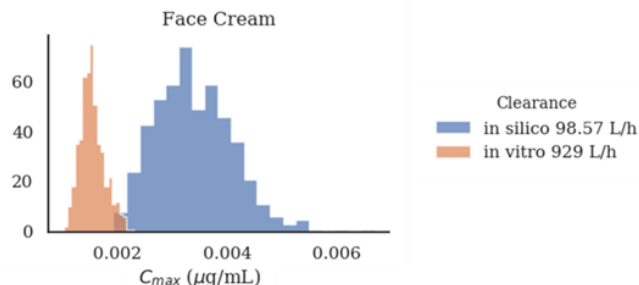
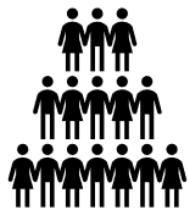
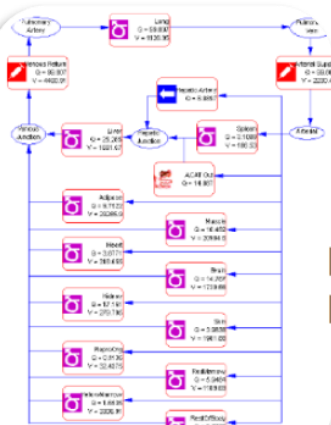


<https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/science-approach-document-bioactivity-exposure-ratio-application-priority-setting-risk-assessment.html>



The key NAMs in our NGRA approach

PBK Modelling



Toxicology in Vitro (2020), 63, 104746

In vitro pharmacological profiling

PERSPECTIVES

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

Joanne Bowes, Andrew J. Brown, Jacques Hamon, Wolfgang Jorntrup, Arun Srihar, 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.

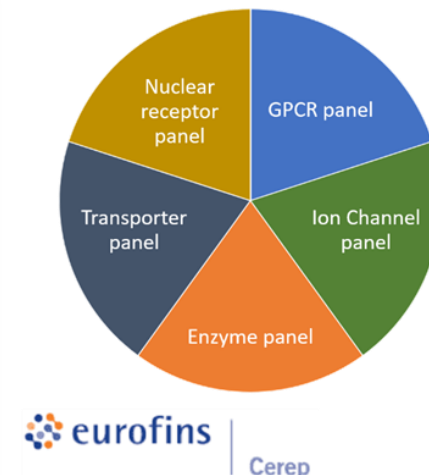
Decreasing the high attrition rate in the drug discovery and development process is a primary goal of the pharmaceutical industry. One of the main challenges in achieving this goal is striking an appropriate balance between drug efficacy and potential adverse effects as early as possible in order to 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 critical for reducing the likelihood of safety issues limiting the use of approved drugs, or even leading to their market withdrawal, bearing in mind the recently revised and reinforced conditions target (or targets), whereas secondary effects are due to interactions with targets other than the primary target (or targets) (that is, off-target interactions). Off-target interactions are often the cause of ADRs in animal models or clinical studies, and so careful characterization and identification of secondary pharmacology profiles of drug candidates early in the drug discovery process might help to reduce the incidence of type A ADRs.

In vitro pharmacological profiling involves the screening of compounds against a broad range of targets (receptors, ion channels, enzymes and transporters) that are known from the intended safety testing of drug candidates and are designed to prevent serious ADRs from occurring in clinical studies.

The only *in vitro* pharmacology assay that is absolutely required by regulatory authorities to ensure that measures the effects of new chemical entities on the ionic current of cardiac L-type heterologously expressed human voltage-gated potassium channel subunit 19 (number 2) (hKv2.1) 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-line 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 include the stage of the discovery process at which an *in vitro* pharmacological profiling should occur. Nevertheless, the general need for most pharmaceutical companies is to perform this testing early in the drug discovery process to reduce attrition and to facilitate better prediction of ADRs in the later stages of drug discovery and development.

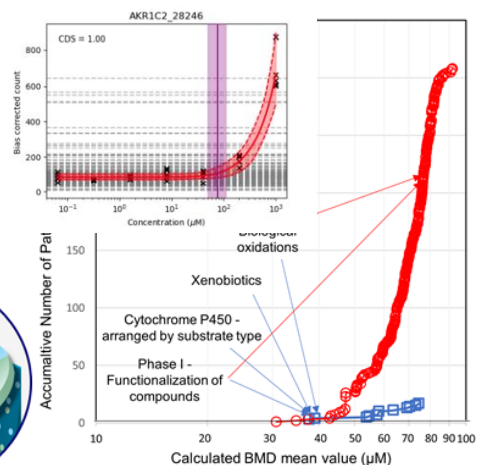
Here, for the first time, four major pharmaceutical companies (AstraZeneca, GlaxoSmithKline, Novartis and Pfizer) share their knowledge and experience of the innovative application of existing screening technology to detect off-target interactions of compounds. The objective of this article is to describe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



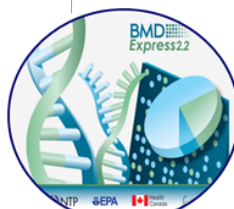
euoifins | Cerep

Transcriptomics

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



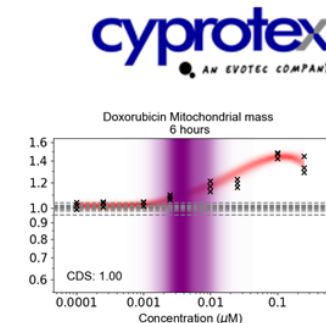
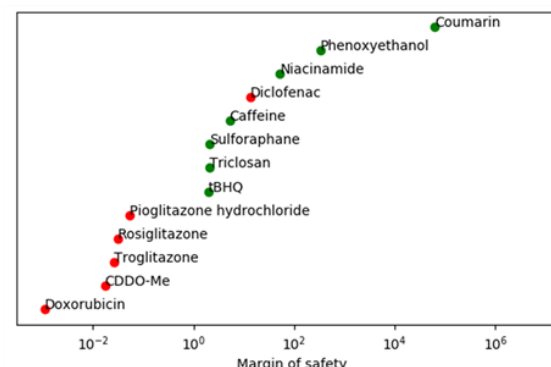
BMDexpress 2
BiFrost



10 stress pathways, 36 Biomarkers 8 concentrations

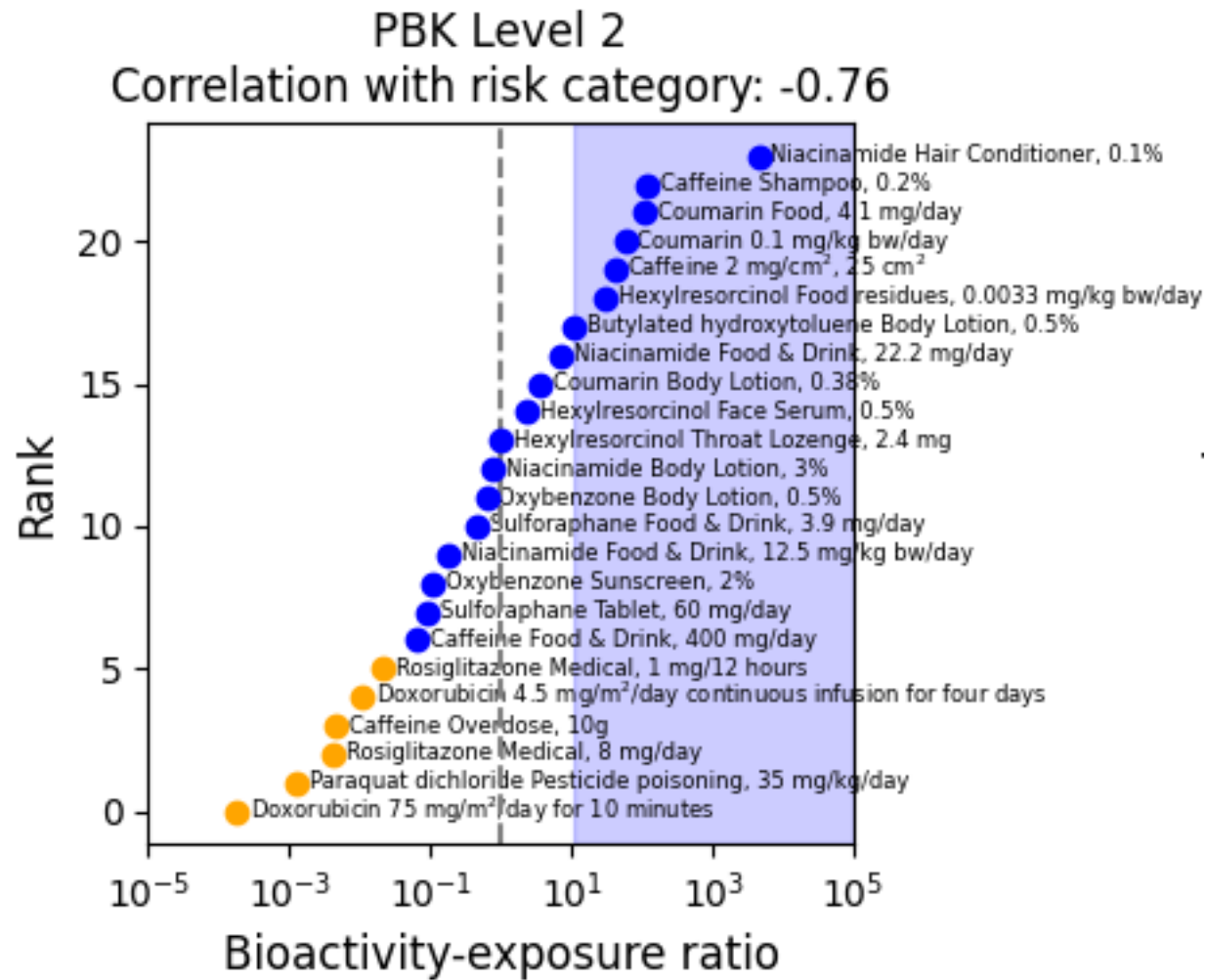
13 chemicals, 36 Biomarkers; 3 Timepoints; 8 Concentrations; ~ 10 Stress Pathways

- 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)
 - Dasturubicin (drug)
 - Diclofenac (drug)
 - Troglitazone (drug)
 - Pioglitazone (drug)
 - Rosiglitazone (drug)



Toxicol Sci (2020), 176, 11-33

Visualising how the toolbox performs against the pilot study data



Blue: low risk chemical-exposure scenario

Yellow: high risk chemical-exposure scenario

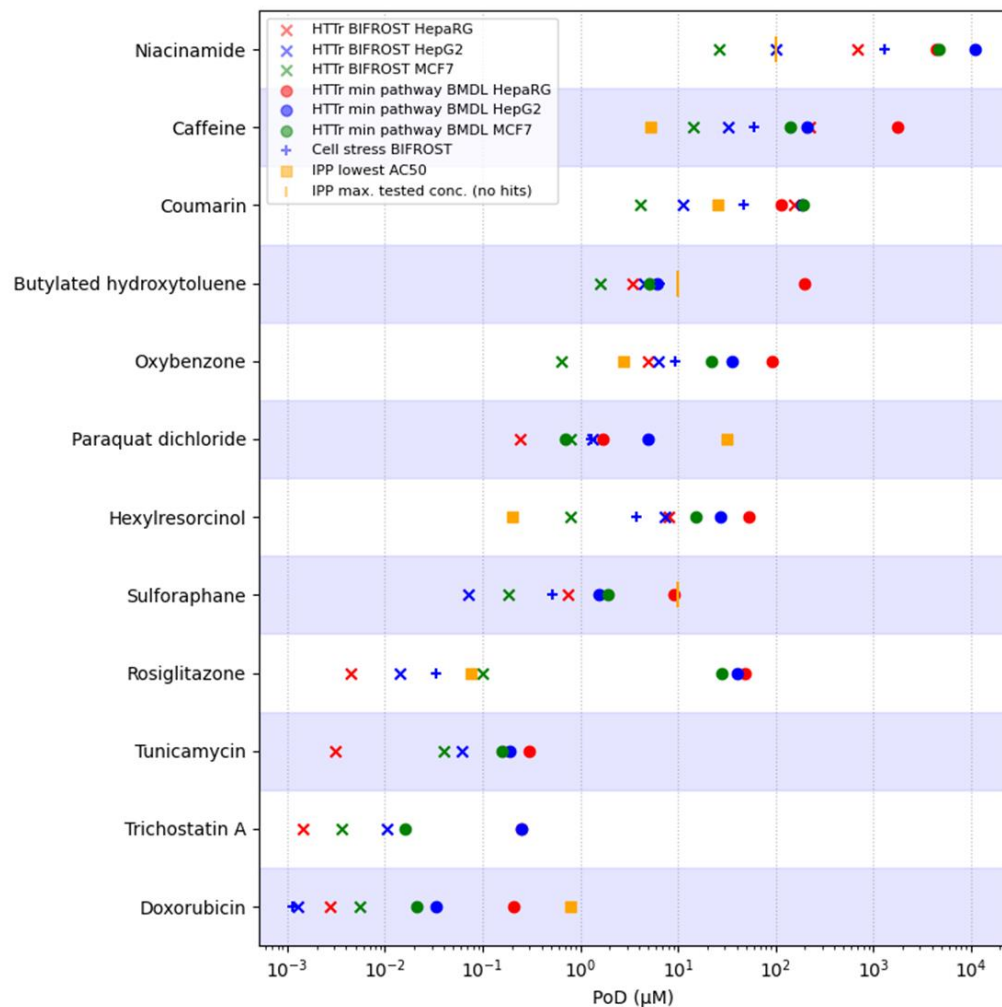
Exposure scenarios within the blue shaded region are identified as low risk.



Are Non-animal Systemic Safety Assessments Protective? A Toolbox and Workflow

Alistair M. Middleton *,¹ Joe Reynolds,* Sophie Cable,*
 Maria Teresa Baltazar,* Hequn Li *, Samantha Bevan,[†] Paul L. Carmichael,*
 Matthew Philip Dent,* Sarah Hatherell,* Jade Houghton,* Predrag Kukic,*
 Mark Liddell,* Sophie Malcomber,* Beate Nicol,* Benjamin Park,[†] Hiral Patel,[‡]
 Sharon Scott,* Chris Sparham,* Paul Walker *,[†] and Andrew White*

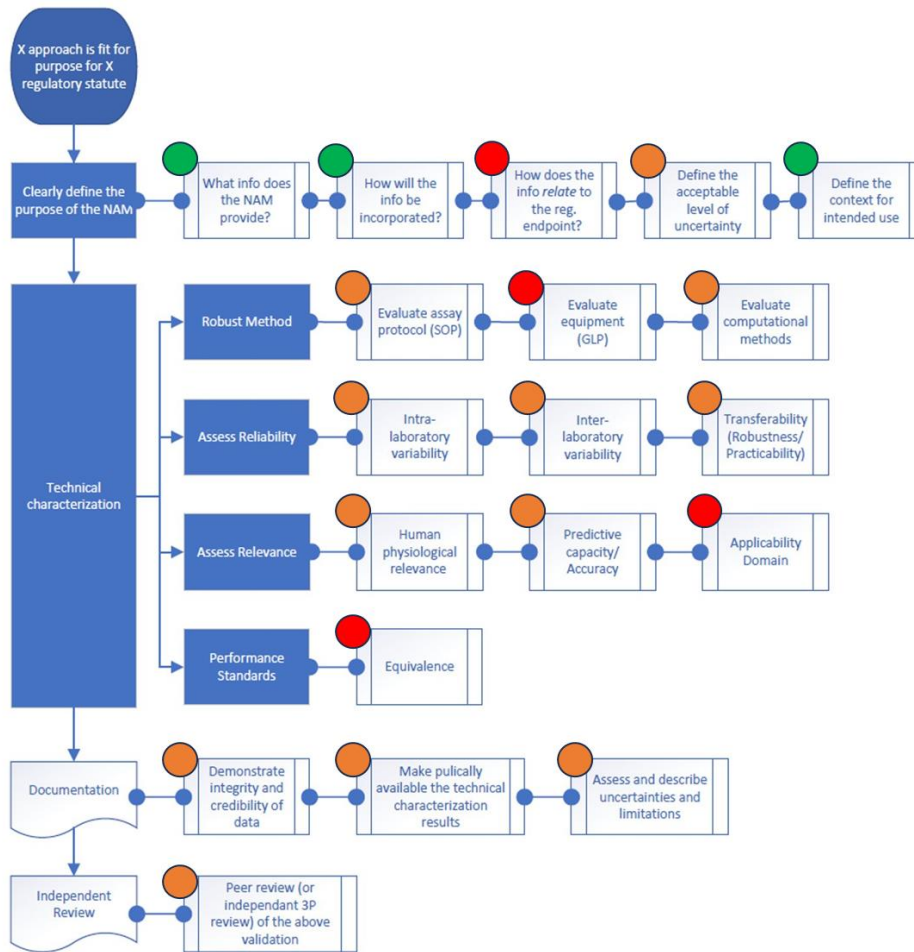
Transcriptomic POD tend to be one of most sensitive especially at a gene level.



- For 60% of compounds tested to date HTTr provides the most conservative (lowest POD)
- As previously observed shifting from a gene level to a pathway based increases the predicted concentration of the POD

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

How do we build scientific confidence in a systemic safety toolbox?



A framework for establishing scientific confidence in new approach methodologies

Anna J. van der Zalm¹ · João Barroso² · Patience Browne³ · Warren Casey⁴ · John Gordon⁵ · Tala R. Henry⁶ · Nicole C. Kleinstreuer⁷ · Anna B. Lowit⁶ · Monique Perron⁸ · Amy J. Clippinger¹

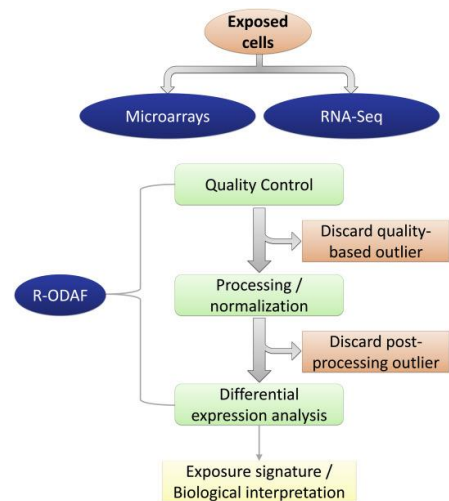
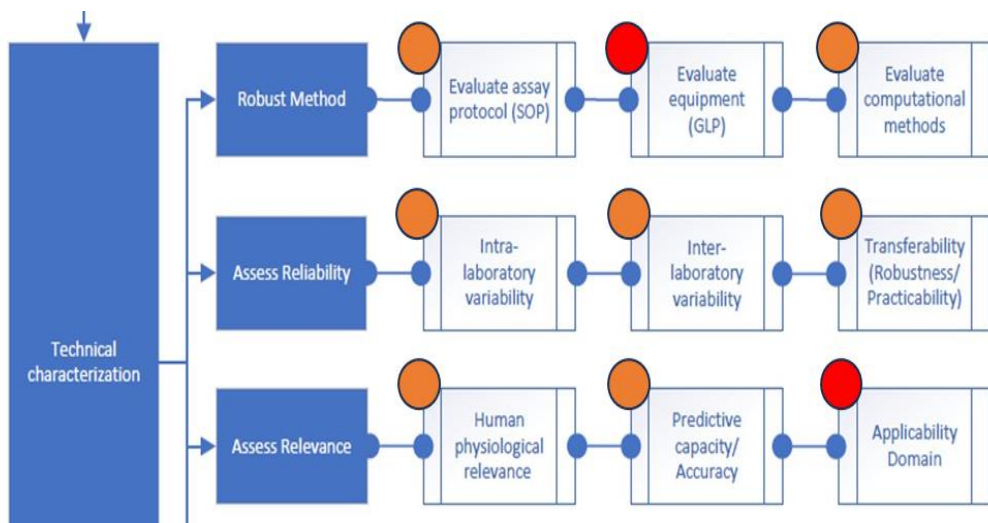
Received: 17 May 2022 / Accepted: 11 August 2022 / Published online: 20 August 2022
© The Author(s) 2022

1. Determine whether the toolbox is fit for purpose.
2. Take into account human safety in assessing the approach (where possible)
3. Identify what an appropriate safety decision might be (e.g., BER threshold).

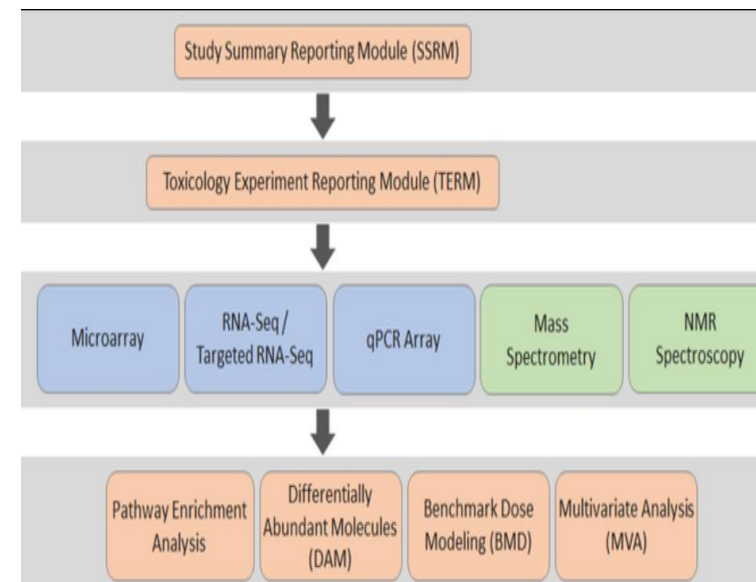


Building Scientific confidence in application of HTTr

Ability to generate reliable and consistent reproduction of results is the prerequisite for successful application of TGx results in the regulatory setting



R-ODAF –Verheijen et al 2022



OECD -ORF –Harrill et al 2021

- Providing supporting evidence to accelerate confidence and acceptance for decision making.

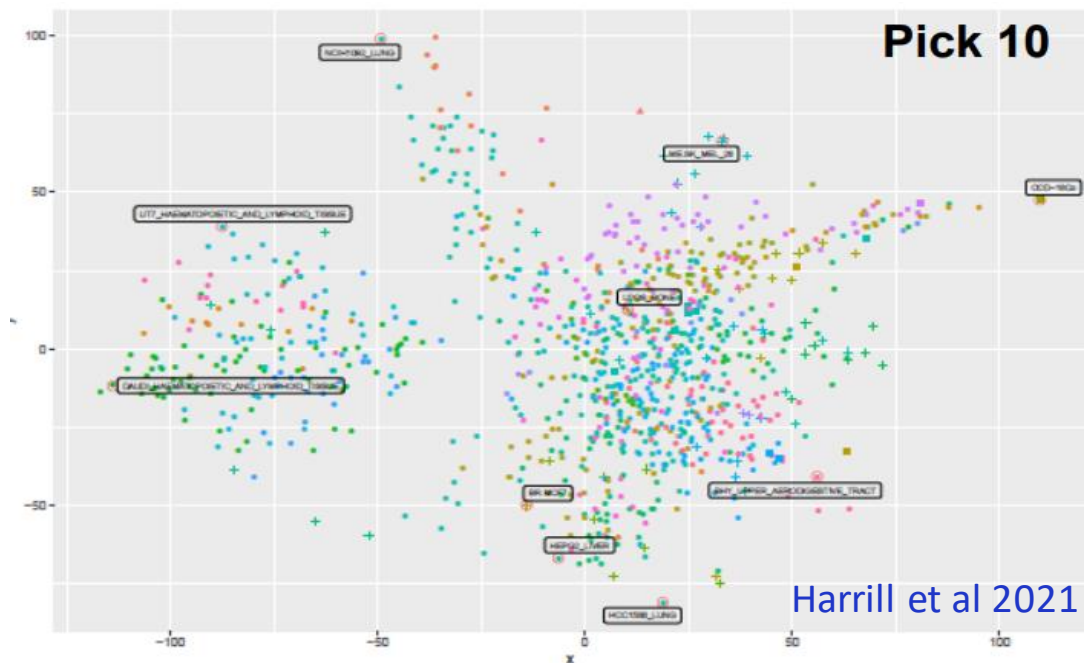
Cellular Models
incl. Treatment

Data Acquisition

Data Modelling

Interpretation

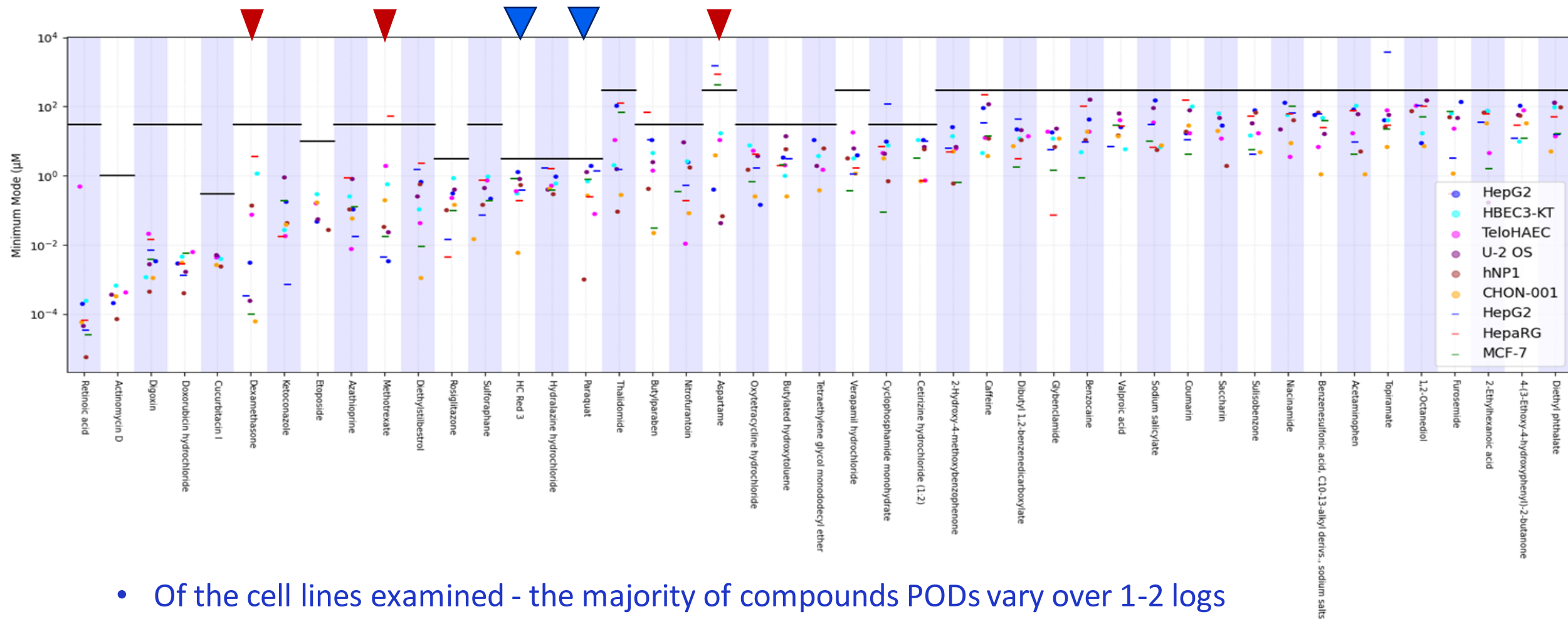
Cell line Applicability



Cell_Type	Tissue_Origin	Cell_Type	Tissue_Origin
U-2 OS	Bone	U-2 OS	Bone
MCF-7	Breast	MCF-7	Breast
HepaRG_2D	Liver	HepaRG_2D	Liver
HBEC3-KT	Lung	CHON-001	Fibroblast
CHON-001	Fibroblast	HBEC3-KT	Lung
TeloHAEC	Vascular	TeloHAEC	Vascular
RPTEC	Kidney	RPTEC	Kidney
ARPE-19	Retina	ARPE-19	Retina
HPNE	Pancreas	HPNE	Pancreas
Ker-CT	Skin	CCD-18Co	Fibroblast
CCD-18Co	Fibroblast	Ker-CT	Skin
ASC52telo	Mesenchymal Stem Cell	ASC52telo	Mesenchymal Stem Cell
BJ-5ta	Fibroblast	BJ-5ta	Fibroblast
HME-1	Breast	RPE-1	Retina
RPE-1	Retina	HME-1	Breast
TIME	Vascular	TIME	Vascular
HUVEC	Vascular	HUVEC	Vascular
HSAEC-1	Lung	HSAEC-1	Lung

- Breadth of coverage of biological pathways - No single cell line captures all biological variation
 - Complexity of surrogate test system compared to integrated systems
 - Acute vs chronic responses / Sensitivity
- Initial cell line use focused on historical use patterns – ie Data availability for gene signature comparisons/ Use in other ongoing assays / Metabolic competency
- Develop Data driven approach to extend and maximise the biological space covered

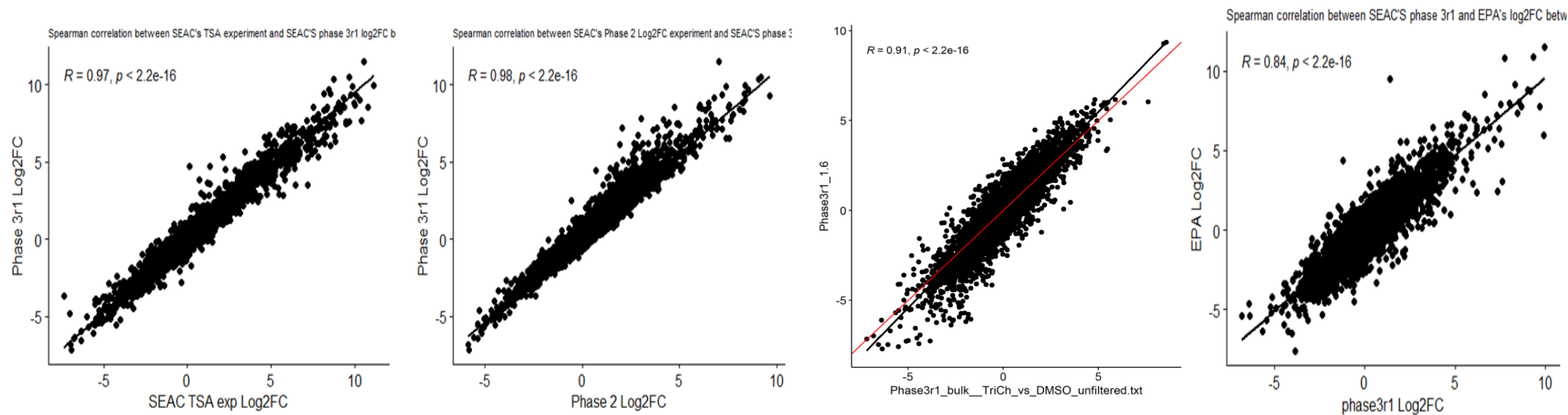
Cell line DR variability following compound treatment



- Of the cell lines examined - the majority of compounds PODs vary over 1-2 logs
- Some cases where this variability extends over a greater range >4 logs ▼
- Few cases where single cell line is significantly more sensitive than the others ▼



- Bulk lysate samples – understand inter run variability of sequencing process
- Fresh positive control samples understand whole process variability including sample generation.
- Ensure assay variability remains within defined limits



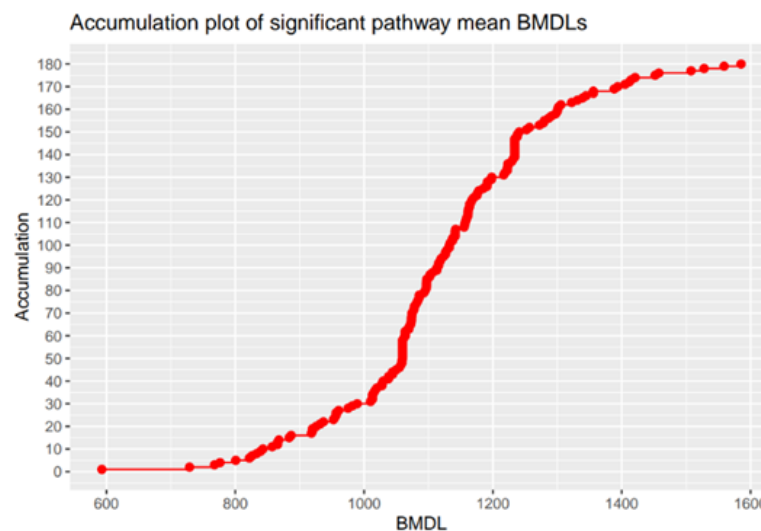
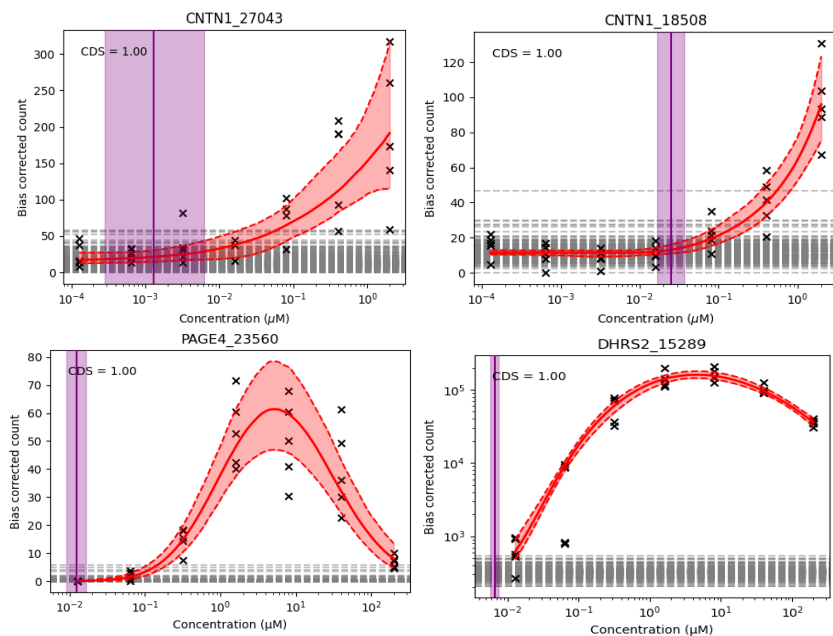
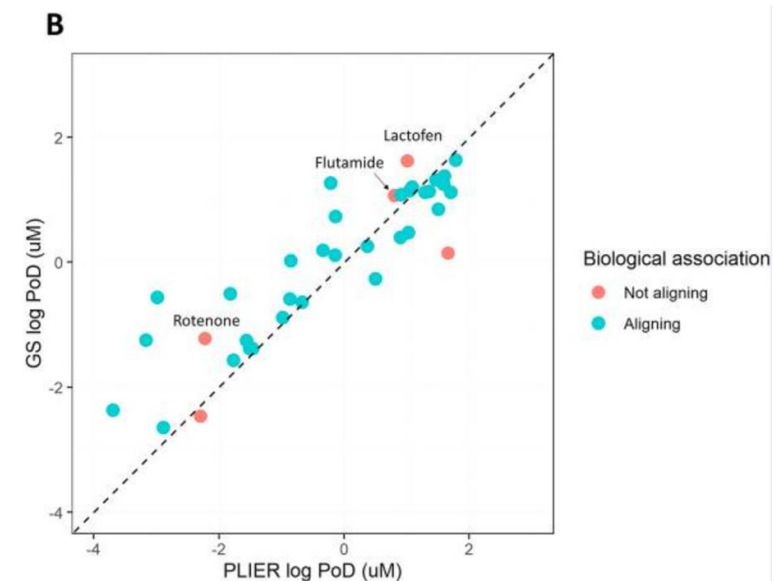


Figure 6: Significant pathway BMDL accumulation plot



Basili et al 2022 Latent Variables Capture Pathway-Level Points of Departure in High-Throughput Toxicogenomic Data
[Chem Res Toxicol.](#) 35: 670–683



Computational Toxicology
 Volume 16, November 2020, 100138

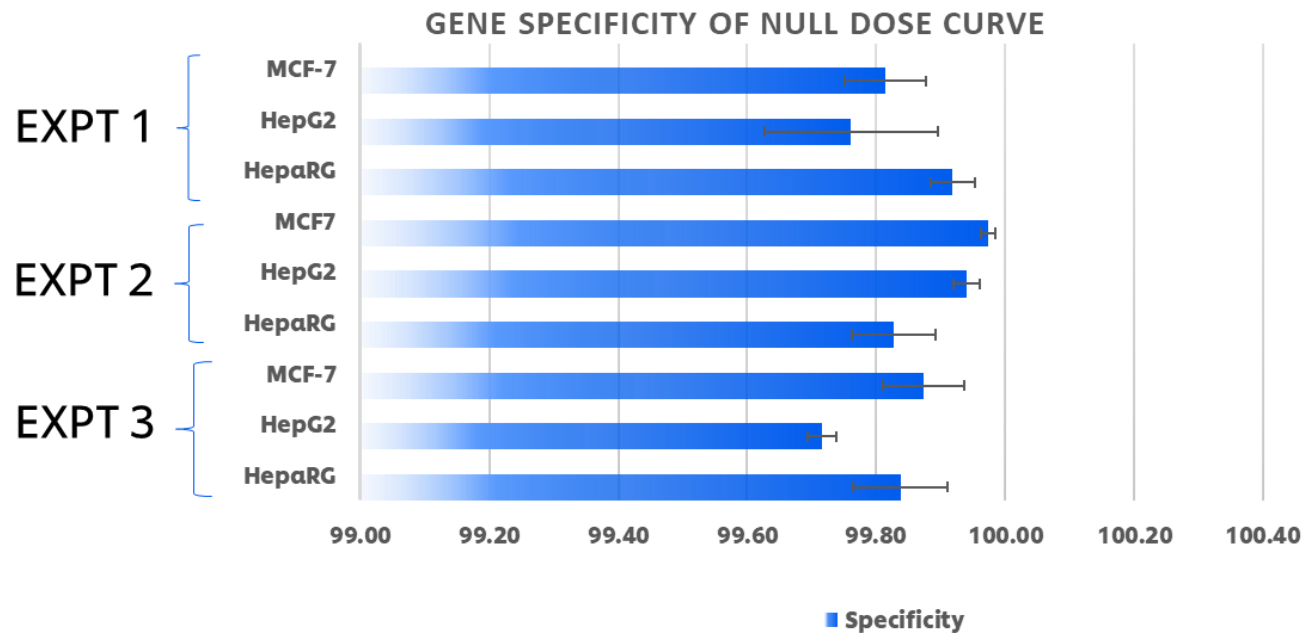


A Bayesian approach for inferring global points of departure from transcriptomics data

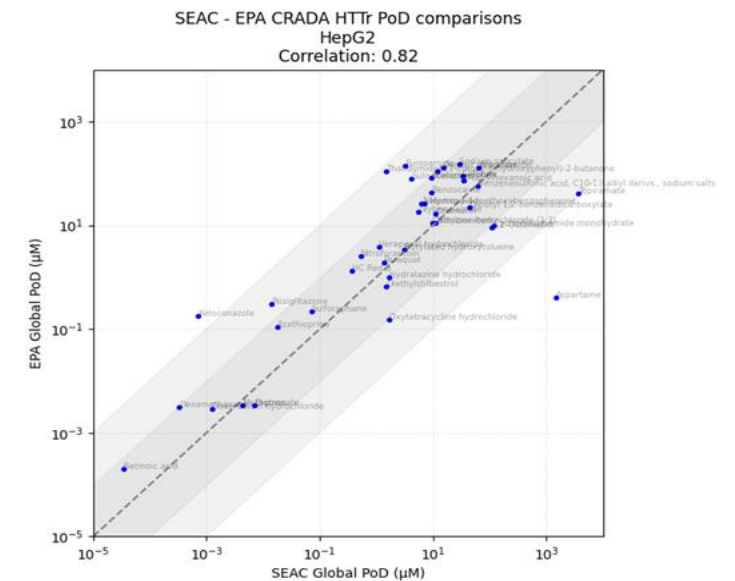
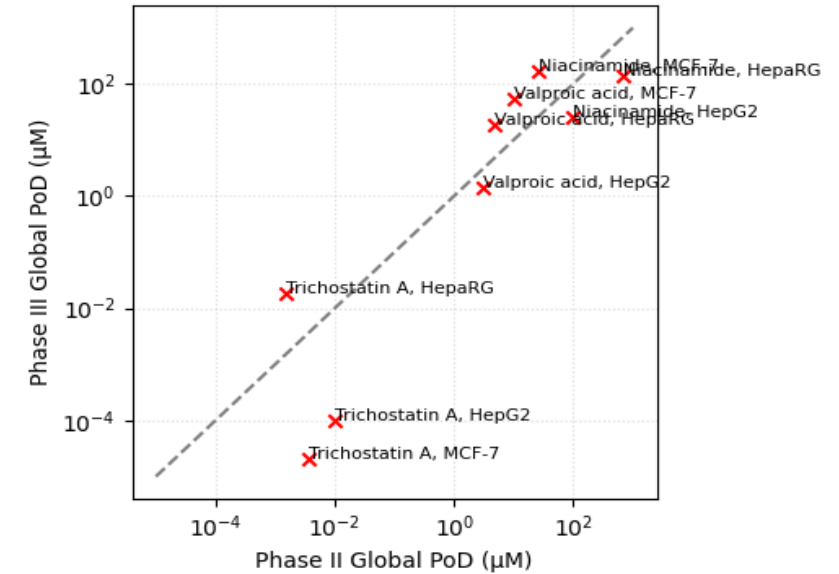
Joe Reynolds, Sophie Malcomber, Andrew White

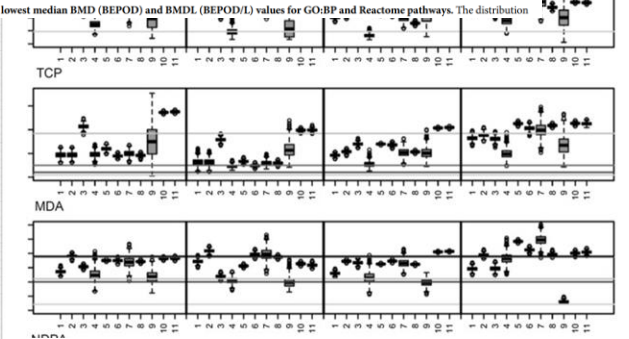
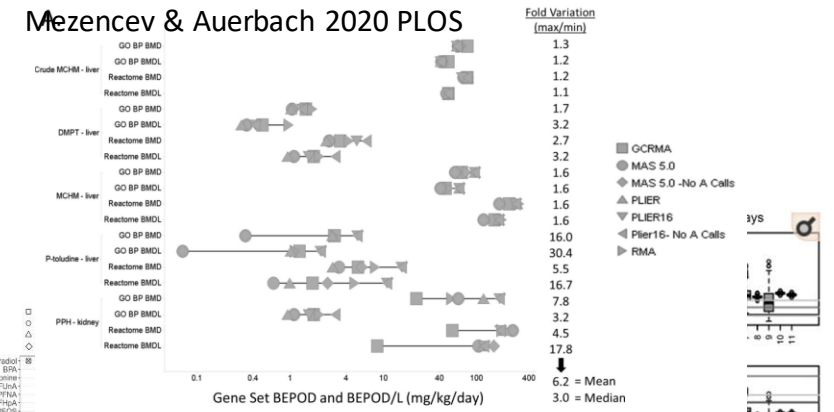
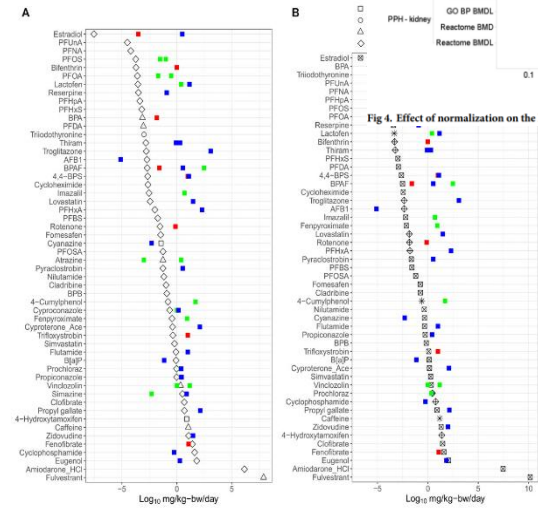
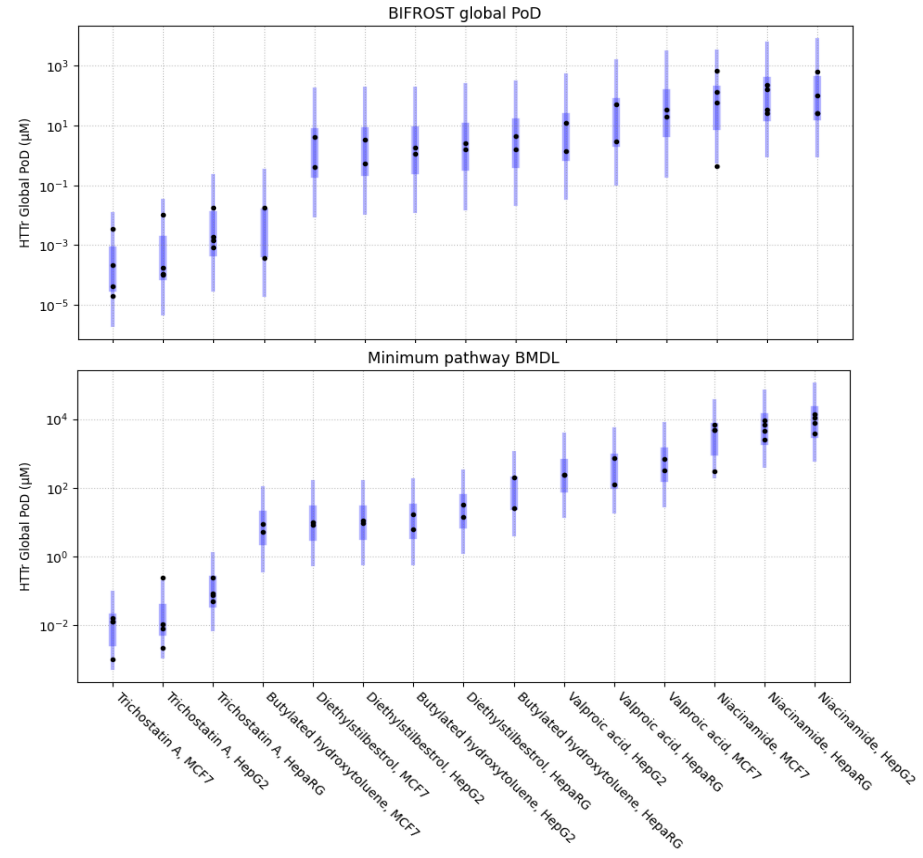
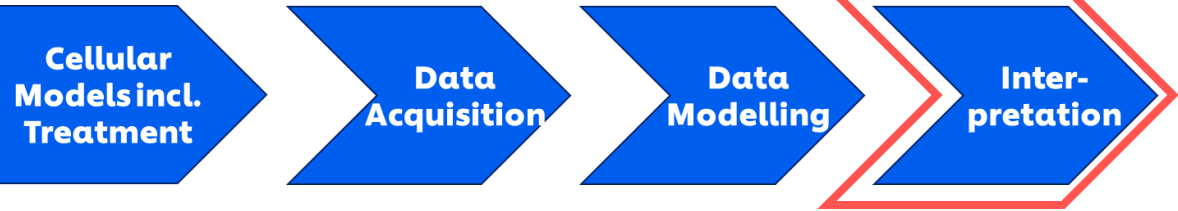


Philips et al 2019 BMDExpress 2: enhanced transcriptomic dose-response analysis workflow
[Bioinformatics](#) 35: 1780-1782



- Application of negative control data to provide a ground truth and estimate false positive predictivity
- Build synthetic data sets of known dose response profiles to estimate positive predictivity
- Reproducibility of derived PODs from replicate experiments (7 out of 9) within 1 log order magnitude





Reardon et al 2023 Front. Toxicol

Farmahin et al. 2017 Arch. Tox

Large area of development and ongoing research to define new approaches for POD estimation
 Use of benchmarking to assess utility



Summary

- Exposure-led approach to determine protection through a BER (MoS) – range of different case studies now showing utility of approach
- Focus on weight of evidence to show tools can be integrated to make a safety decision - requires diverse expertise
- Strength derived from integrating a combination of targeted and broad unbiased tools – not a one to one replacement
- Utilise NAMs for further targeted follow where required to refine uncertainty e.g. metabolism
- NAMs not standardised - need to ensure robustness/quality of tools and include estimations of uncertainty to aid acceptance
- Further activity required to build evaluation data sets and ground truth to evaluate current approaches and those in the future
- Collaboration required to progress assessment and build out confidence for broader stakeholder community on applicability domains/ remaining gaps

Acknowledgements

Unilever: Maria Baltazar, Sophie Cable , Alistair Middleton, Joe Reynolds, Georgia Reynolds, Beate Nicol, Sharon Scott, Sophie Malcomber, Annabel Rigarlsford, Chris Sparham, Katarzyna Przybylak, Predrag Kukic, Georgia Reynolds, Tom Moxon, Hequn Li, Dawei Tang, Jayasujatha Vethamanickam, Matthew Dent, Paul Carmichael, Sarah Hatherell, Richard Cubberley, Carl Westmoreland

US-EPA: Richard Judson, Josh Harrell, Logan Everett, Imran Shah



Thank You



seac.unilever.com

