

# Development and Application in the use of NAMs for Next Generation Risk Assessment

## Learnings from Industry case studies

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Unilever

# Outline

- **What is NGRA?**
- **Examples of how it could be applied?**
- **How Protective is this?**

# The need for non-animal approaches



Societal Attitudes/Consumer Preference



Scientific Relevance

22.12.2009 EN Official Journal of the European Union L 342/59

**REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 30 November 2009 on cosmetic products (recast) (Text with EEA relevance)**

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty establishing the European Community, and in particular Article 95 thereof,

Having regard to the proposal from the Commission,

Having regard to the opinion of the European Economic and Social Committee (1),

Acting in accordance with the procedure laid down in Article 2 of the Treaty (2),

Whereas:

(1) Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products (3) has been significantly amended on several occasions. Since further amendments are to be made, in this particular case it should be recast as a Regulation.

(5) The environmental concerns that substances used in cosmetic products may raise are considered through the application of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and establishing a European Chemicals Agency (4), which enables the assessment of environmental risks in a cross-sectoral manner.

Is it safe?

NOAEL

Safe Dose in Humans

NOAEL + 10 - 1000

Targeted Testing

Species Extrapolation

Adverse Organism Response

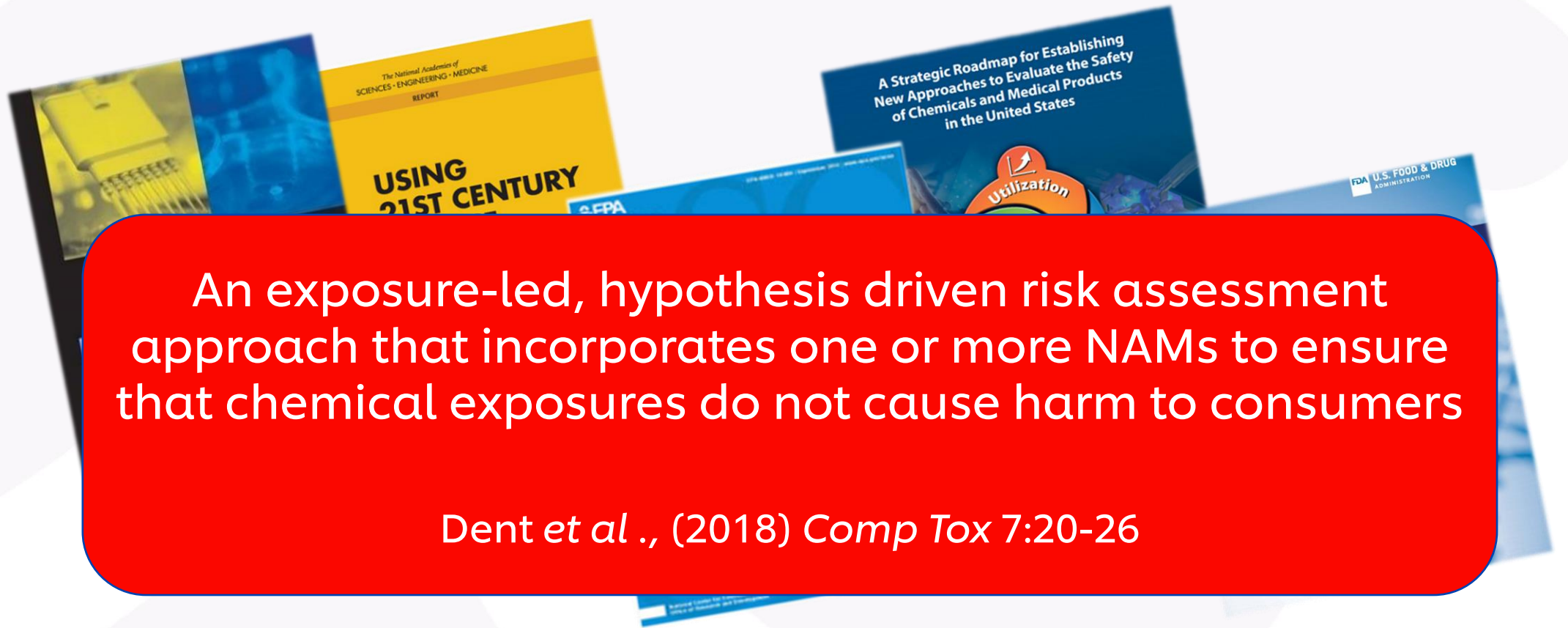
Amount Conc. of ingredient due to exposure

Uncertainty Factors

e.g. 90 Day Repeat Dose Study

Regulatory Change

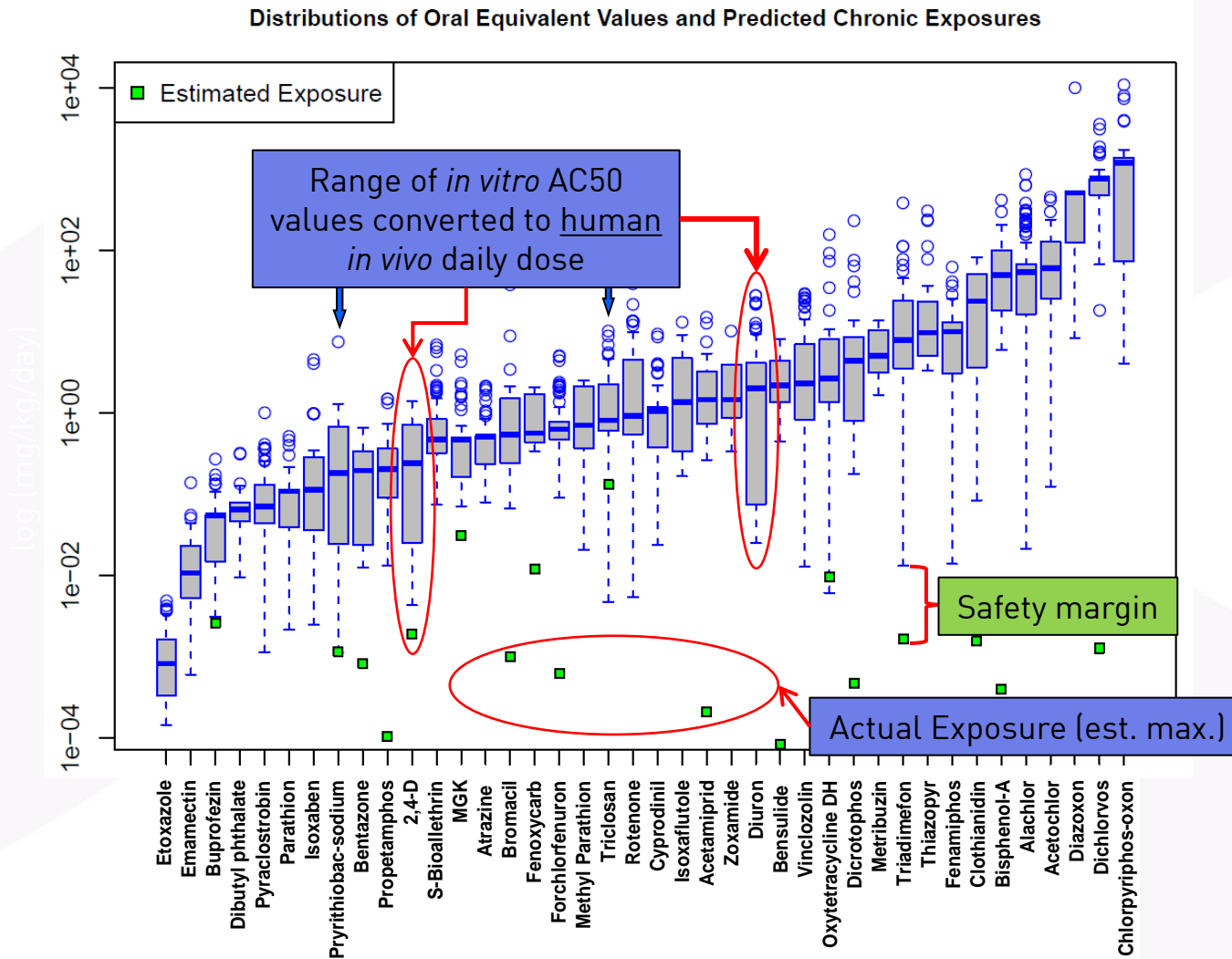
# What is NGRA?



An exposure-led, hypothesis driven risk assessment approach that incorporates one or more NAMs to ensure that chemical exposures do not cause harm to consumers

Dent et al ., (2018) *Comp Tox* 7:20-26

# 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.**



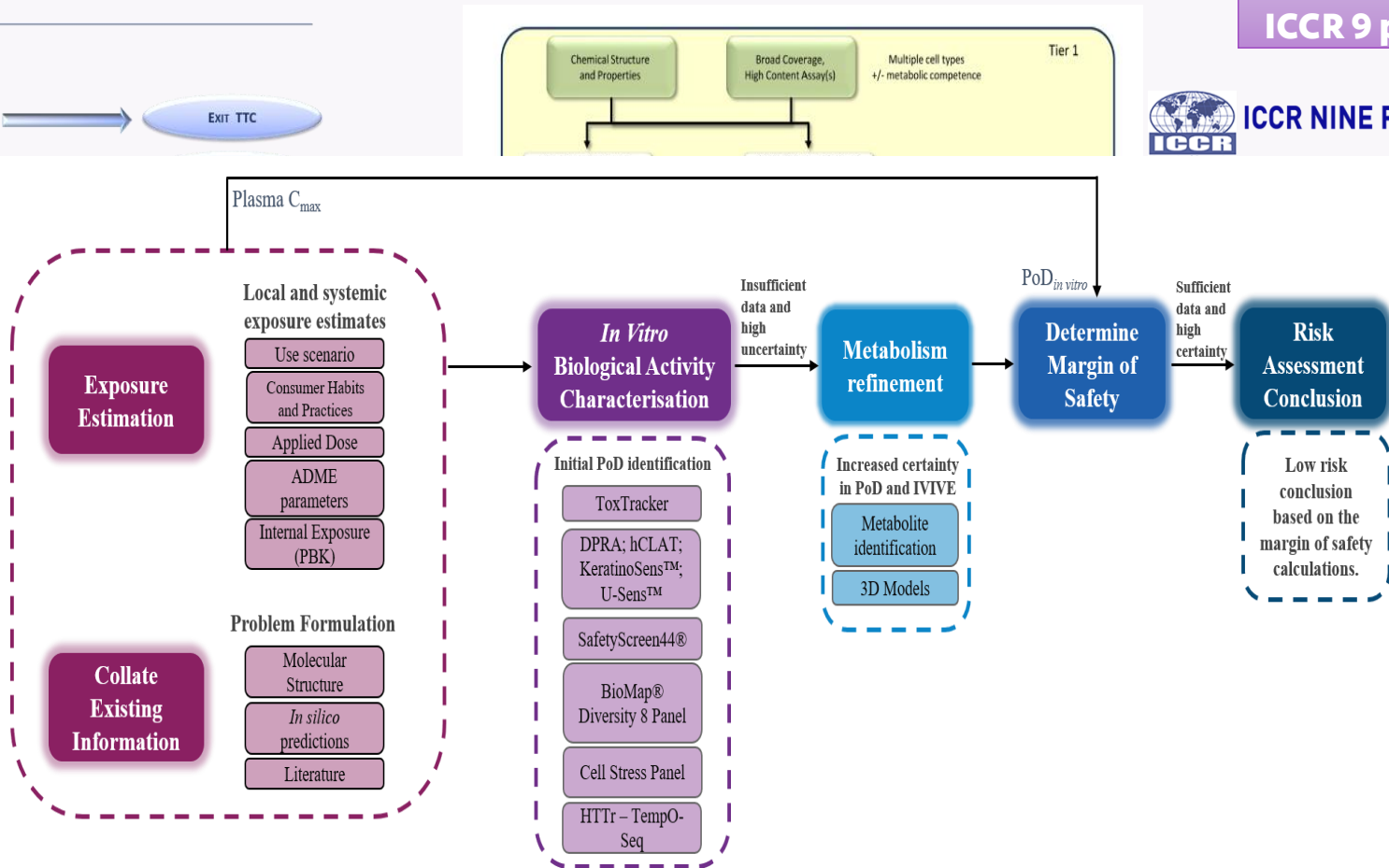
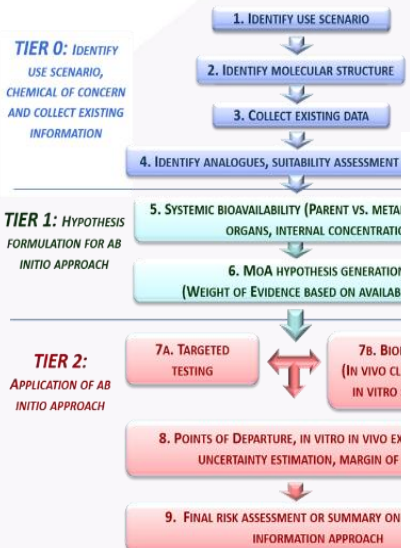
Slide from Dr Rusty Thomas, EPA, with thanks

Rotroff, et al. Tox.Sci 2010

Thomas RS et al., 2019. Tox Sci. 1;169(2):317-332.



# Framework Approach: The overall goal is a human safety risk assessment



## ICCR 9 principles of NGRA



## ICCR NINE PRINCIPLES OF NGRA

- principles:**
  - is a human safety risk assessment
  - is exposure led
  - is hypothesis driven
  - is designed to prevent harm
- define how a NGRA should be conducted:**
  - appropriate appraisal of existing information
  - iterative approach
  - relevant methods and strategies
- documenting NGRA:**
  - uncertainty should be characterized and documented
  - approach should be transparent and documented

Dent et al. 2018 Computational Toxicology, 7, 20-26.

# Case Study approach – Human Health Safety Assessment required for ...

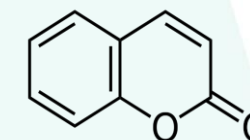
## 0.1% COUMARIN IN FACE CREAM

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

### 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

Problem Formulation



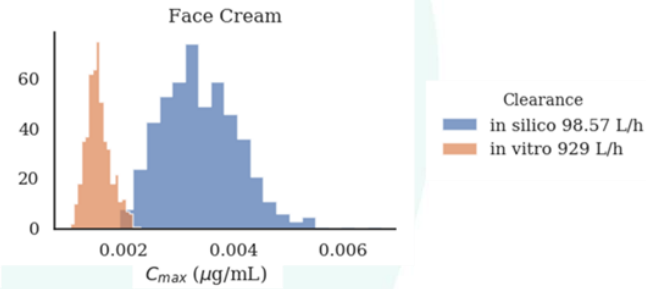
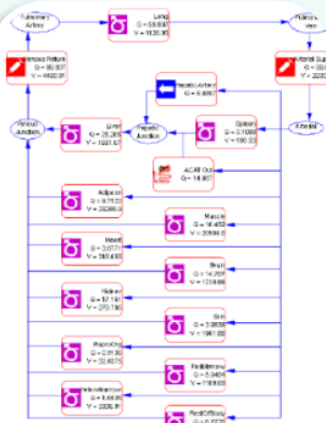
Exposure Led

All safety assessments of cosmetic ingredients are exposure-driven:

Baltazar *et al.*, (2020) *Tox Sci* (vol 176: 236–252)  
<https://doi.org/10.1093/toxsci/kfaa048>

# Some key elements in the NGRA toolbox

## 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 Homan, Wolfgang Juratnik, 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 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, having no impact on the patient's safety and regulatory compliance.

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 careful characterization and identification of secondary pharmacology profiles of drug candidates early in the drug discovery process might help reduce the incidence of type A ADRs.

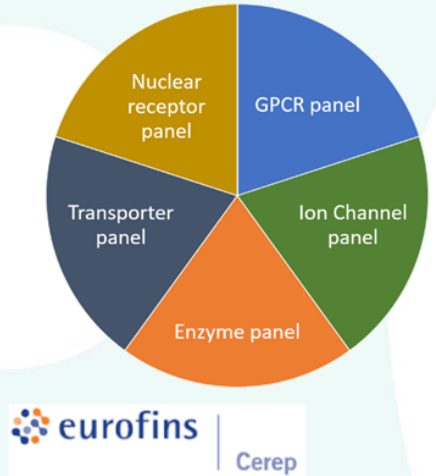
In vitro pharmacological profiling involves the screening of compounds against a broad range of targets (receptors, ion channels, enzymes and transporters) that are chosen from the scientific

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 ion channels of human voltage-gated potassium channels subfamily II member 2 (KCNH2), also known as hERG. The mechanism by which blockade of hERG can affect potentially fatal cardiac arrhythmias (torsades de pointes) following a prolongation of the QT interval is well characterized<sup>1,2</sup>, and the assessment 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 entities<sup>3</sup>.

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 trend for most pharmaceutical companies is 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 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 strategies for the use of an *in vitro* pharmacological profiling panel to reduce drug attrition and to

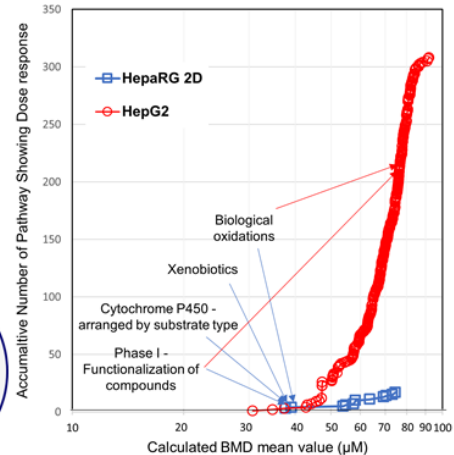
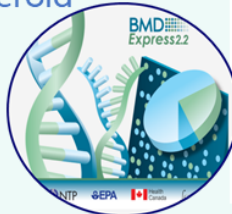


eurolins | Cerep

## Transcriptomics

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

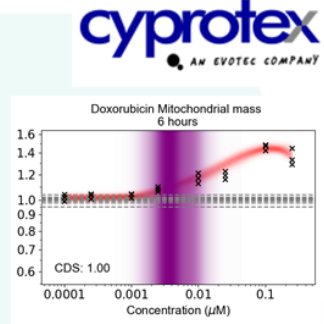
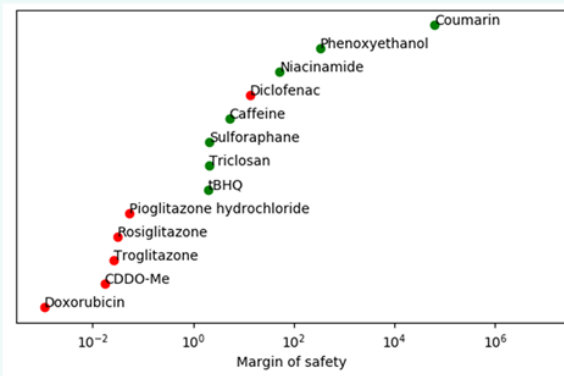
BMDexpress 2



## Cellular Stress Pathways

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

- Exposure scenario adopted for chemical is 'low risk'** (from consumer goods perspective)
- Niacinamide [food, cosmetics]
  - Caffeine [beverages, cosmetics]
  - Phenoxyethanol [cosmetics]
  - Sulfonaphane [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]



Toxicol Sci (2020), 176, 11-33



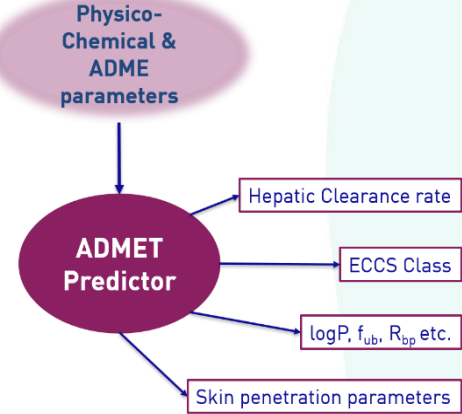
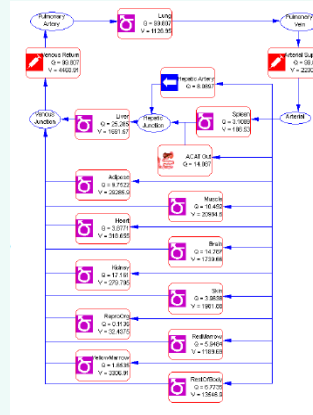
# NGRA for 0.1% coumarin in face cream: exposure estimation

Exposure Estimation

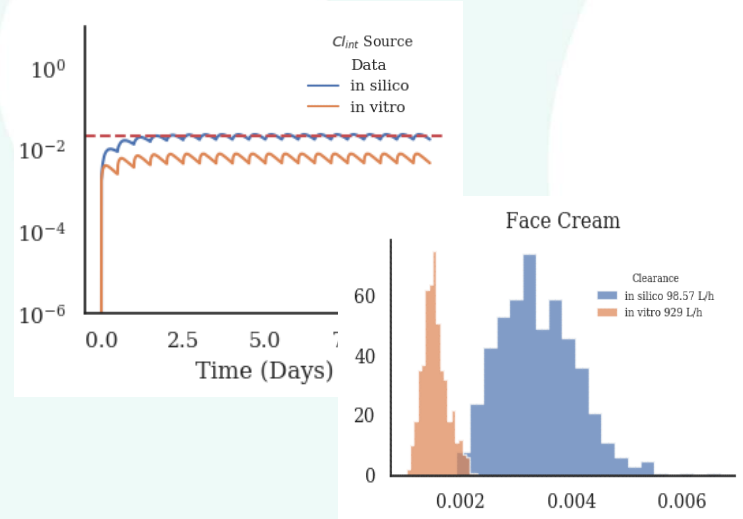
Local and systemic exposure estimates

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

## GastroPlus® (Simulations Plus)



## Level 2- Simulated plasma concentration of coumarin after dermal exposure.



## Level 2. Uncertainty and population variability Distribution of C<sub>max</sub> values after performing Monte Carlo simulation.

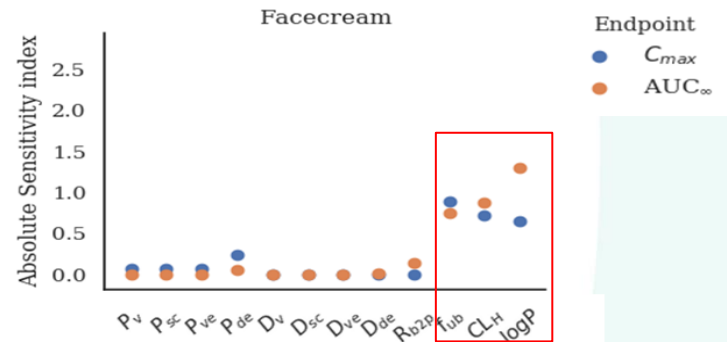
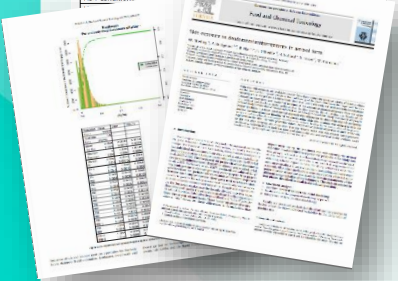


Table 2: Estimated daily exposure levels for different cosmetic product types according to Consumer Europe data (SCENARIOS 1/01; Hall et al., 2007, 2011).

Product type	Estimated daily amount applied (mg/d)	Relative absorption (%)	Relative exposure (mg/d)	Calculated daily exposure (mg/d)	Relative exposure (mg/d)
<b>Bathing, showering</b>					
Shower gel	18.87 g	279.23	0.01	0.19	2.79
Hand water soap	70.00 g	0.01	0.20	1.33	
<b>Hair care</b>					
Conditioner	10.11		0.11	1.51	



B. Hall et al./Food and Chemical Toxicology 49 (2011) 408–422

Total Plasma C <sub>max</sub> (μM)	Mean	Median	90th percentile	95th percentile	97.5th percentile	99th percentile
Face Cream	0.0022	0.0021	0.004	0.0043	0.0046	0.005

**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

# NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: In vitro binding and enzymatic assays: Eurofins SafetyScreen44

**To investigate possible interactions between coumarin and the 44 key targets involved in drug attrition**

## PERSPECTIVES

**A GUIDE TO DRUG DISCOVERY — OPINION**

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

Joanne Bowes, Andrew J. Brown, Jacques Hamon, Wolfgang Jarolimek, Arun Sridhar, Gareth Waldron and Steven Whitebread

**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<sup>1</sup> 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, bearing in mind the growing societal and regulatory emphasis

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.

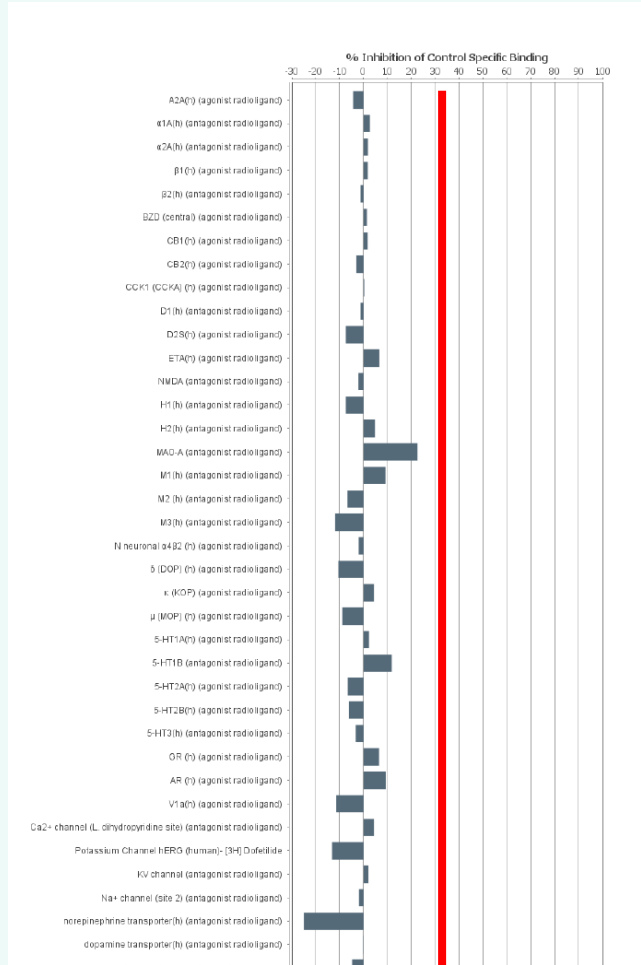
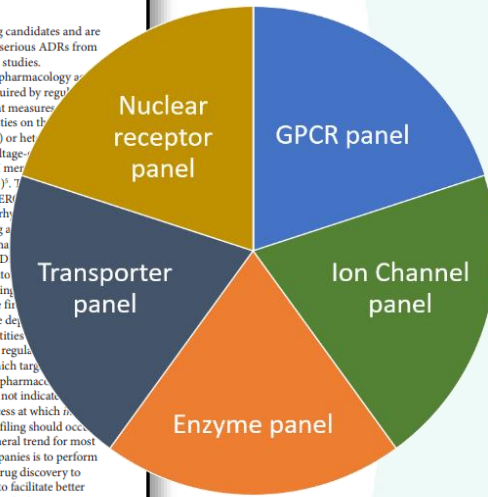
*In vitro* pharmacological profiling involves the screening of compounds against a broad range of targets (receptors, ion channels, enzymes and transporters) that are distinct from the intended

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 is one that measures the current of native ( $I_{Na}$ ) or heterologously expressed human voltage-gated sodium channel subfamily H member 1 (hNav1.5), also known as hERG<sup>2</sup>, which blockade of hERG<sup>2</sup> is a typically fatal cardiac arrhythmia (long QT interval) following a QT interval is well characterized. The seriousness of this ADR is such that this assay is a mandatory part of the assessment of the drug safety of novel chemical entities.

However, current regulatory requirements do not describe which targets should constitute an *in vitro* pharmacological profiling panel and does not indicate the extent of the discovery process at which *in vitro* pharmacological profiling should occur. Nevertheless, the general trend for most pharmaceutical companies is 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 knowledge and experiences 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



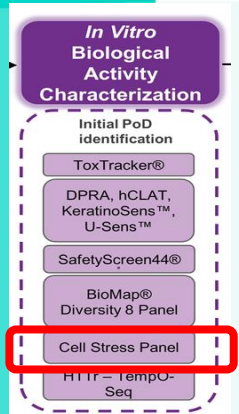
**Results:**

**All binding and enzymatic assay results were negative at 10 μM**

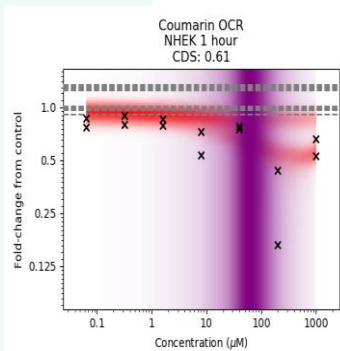
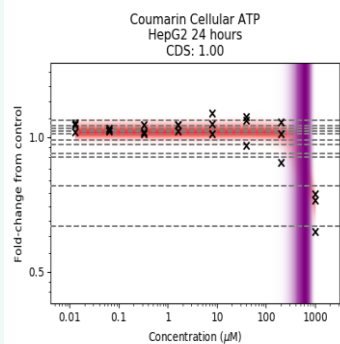


# In vitro biological activity characterisation: In vitro cell stress panel

- Cellular stress response assays are useful to **characterize non-specific biological activity** which is not mediated via a specific protein/receptor interaction
- Measures a range of biomarkers covering **~10 cell stress pathways**
- Single exposure; 8 concentrations; 1h, 6h & 24hr timepoints; HepG2 & NHEK cells

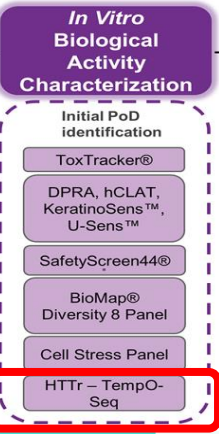


- **Mitochondrial Toxicity:** MitoSOX, PGC1 $\alpha$ , MMP, ATP, Glu/Gal
- **Oxidative Stress:** GSH, ROS, SRXN1, NRF2
- **DNA damage:** pH2AX, p53
- **Inflammation:** TNFAIP3, ICAM1, NFkB p65, IL-1 $\beta$ , IL-8, HMGB1
- **ER Stress:** PERK, ATF4, CHOP, XBP1, BiP, ER Tracker
- **Metal Stress:** MTF-1, Metallothionein
- **Osmotic Stress** (NFAT5);
- **Heat Shock** (HSP70);
- **Hypoxia** (HIF1 $\alpha$ )
- **Cell Health:** LDH, Phospholipidosis, Steatosis, pH rodo indicator, apoptosis (caspase-3/7) & necrosis (ToPro-3)



Biomarkers	Cell type	Stress pathway	PoD (µM)	Effect	Concentration dependency score (CDS)
<b>ATP (6h)</b>	HepG2	cell health	794 (363-977)	down	0.98
<b>ATP (24h)</b>	HepG2		617 (282-891)	down	1
<b>Phospholipidosis (24h)</b>	HepG2	cell health	759 (437-977)	down	0.93
<b>GSH (24h)</b>	HepG2	oxidative stress	851 (301-1000)	up	0.92
<b>IL-8 (24h)</b>	HepG2	inflammation	912 (575-1000)	down	0.61
<b>OCR (1h)</b>	NHEK	mitochondrial toxicity	62 (2.6-776)	down	0.6
<b>OCR (6h)</b>			468 (214-794)		1
<b>OCR (24h)</b>			309 (138-1000)		0.52
<b>Reserve capacity (1h)</b>	NHEK	mitochondrial toxicity	44 (23-96)	down	1
<b>Reserve capacity (6h)</b>			759 (302-1000)		0.9
<b>Reserve capacity (24h)</b>			794 (295-1000)		0.55

Hatherell et al., 2020, <https://doi.org/10.1093/toxsci/kfaa054>



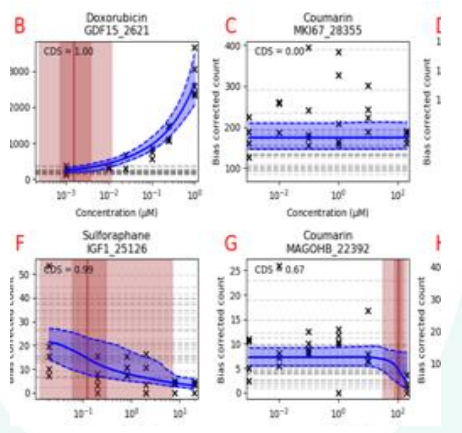
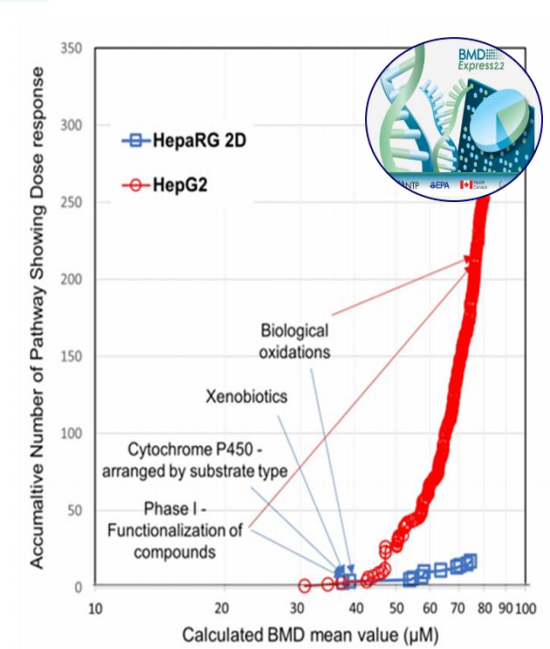
# In vitro biological activity: High-Throughput Transcriptomics (HTTr)

Provide screen for biological activity across a broad biological coverage

- *Tempo-Seq*
- *Human gene panel ver1 ~ 21k*
- *3 cell lines*

**Results:**

- The MCF7 PoD<sub>T</sub> were not considered to be sufficiently robust to derive a MoS
- The lowest PoD<sub>T</sub> for each cell model was selected for the MoS calculation

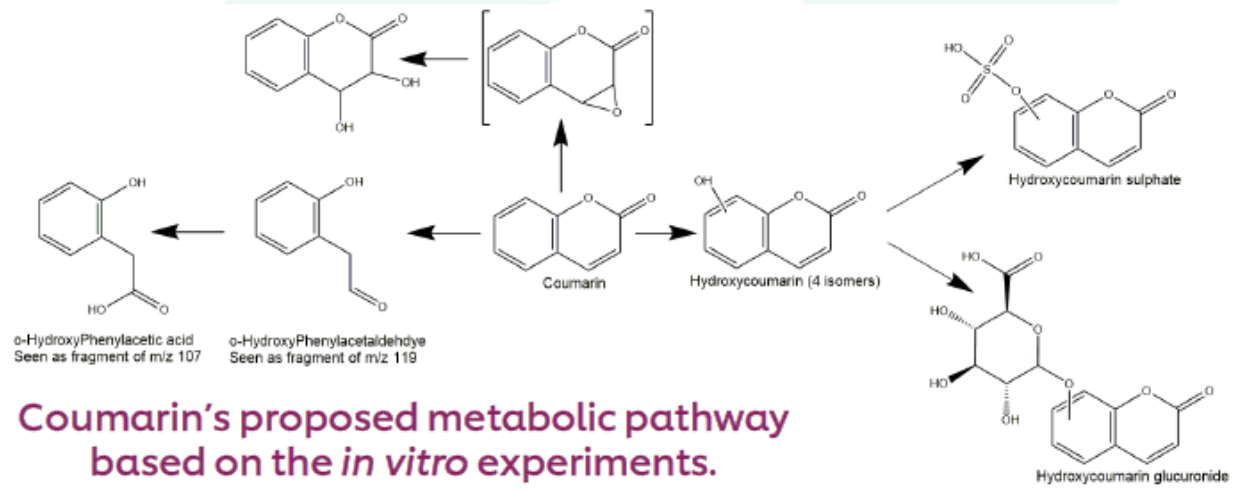
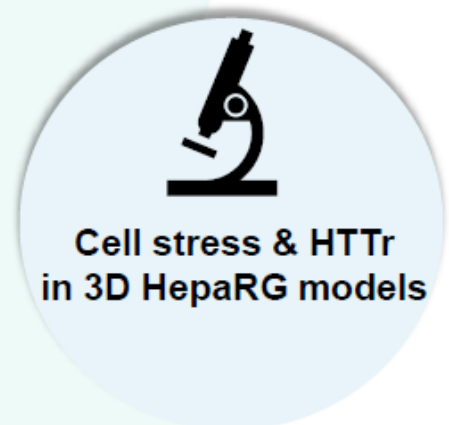
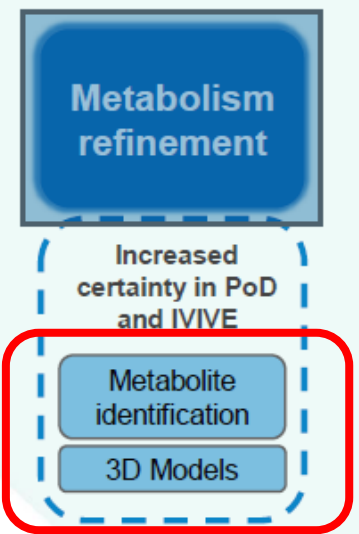


Cell model	HepG2	MCF7	HepaRG 2D
Pathway level tests PoD <sub>T</sub> (µM)	(308 pathways)	(0 pathways)	(17 pathways)
20 pathways with the lowest p value Reactome	70	NA	58*
20 pathways with the lowest BMD Reactome	44	NA	58*
BMD of Reactome pathway with lowest BMD that meets significance threshold criteria	31	NA	38
Gene level tests PoD <sub>T</sub> (µM)	(1570 genes)	(47 genes)	(87 genes)
Mean BMD of 20 genes with largest fold change	6	3	54
Mean BMD of genes between 25 <sup>th</sup> and 75 <sup>th</sup> percentile	17	1	59





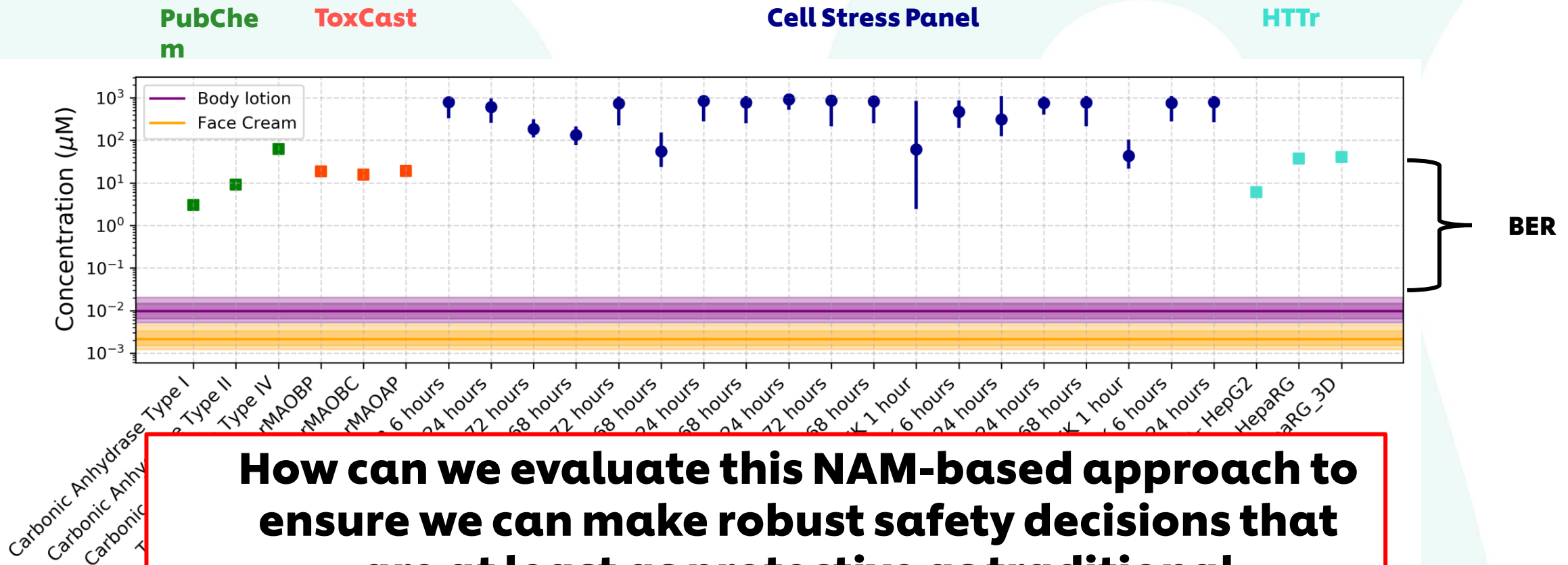
# Tier 2 refinement: Metabolism prediction and activity



- Low bioactivity also found in a metabolic competent cell model (HepaRG 3D)
- PoDs range: 41-871  $\mu\text{M}$  – not very different from 2D cells



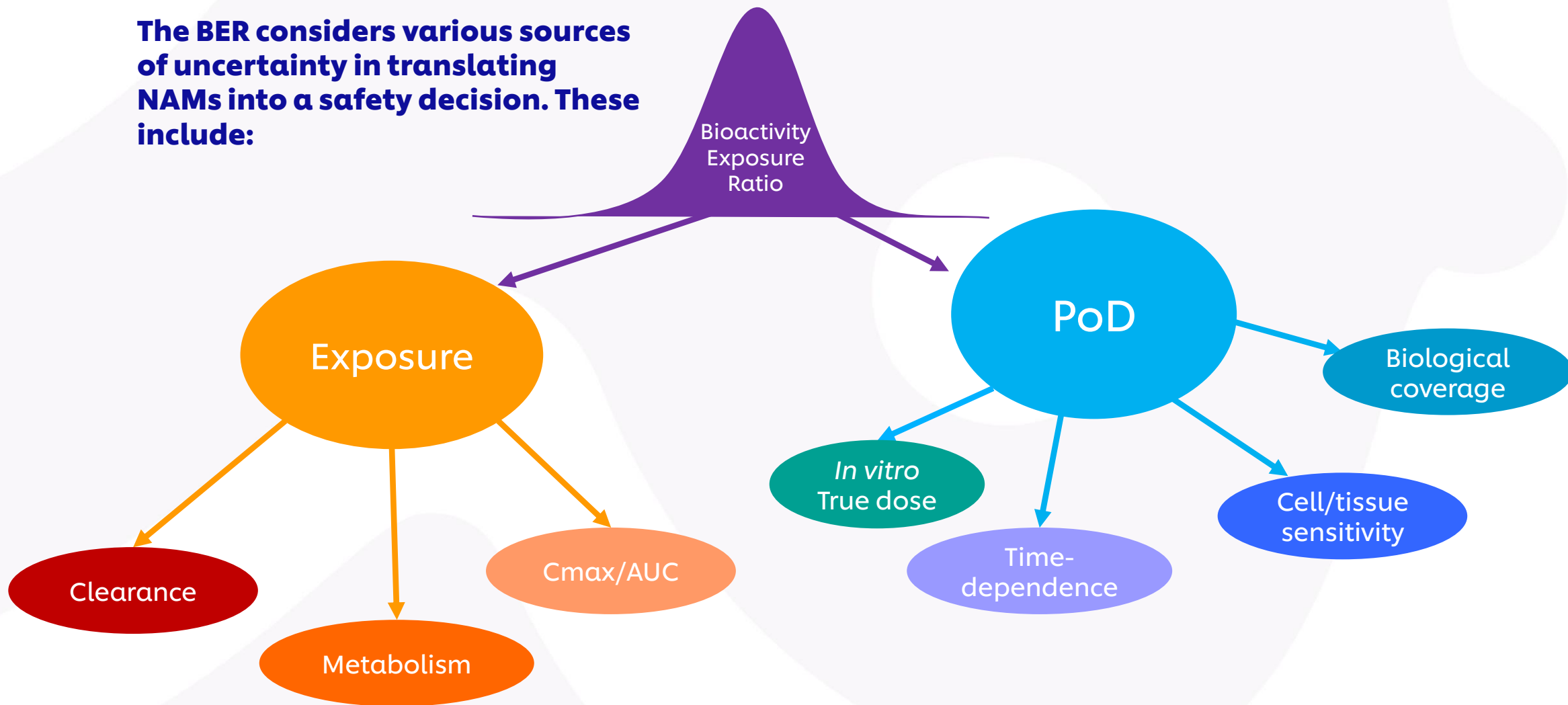
# Exposure and PoD are plotted and used to derive a Bioactivity-Exposure Ratio (BER)



**How can we evaluate this NAM-based approach to ensure we can make robust safety decisions that are at least as protective as traditional approaches**

# Integrating Exposure and Bioactivity Data from NAMs to Make Safety Decisions

The BER considers various sources of uncertainty in translating NAMs into a safety decision. These include:



# How protective are the NAMs?

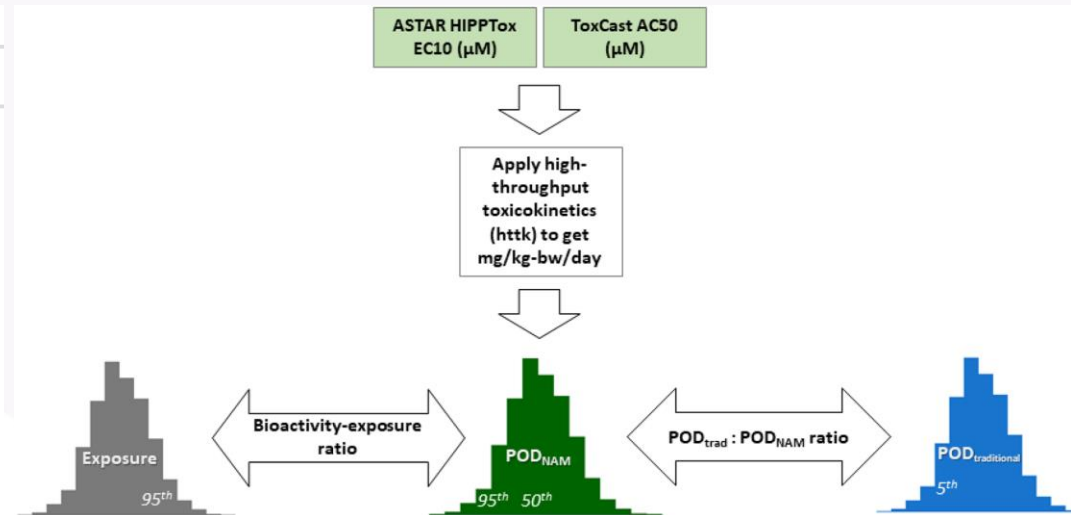
## Example from the Accelerating the Pace of Chemical Risk Assessment (APCRA) initiative



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

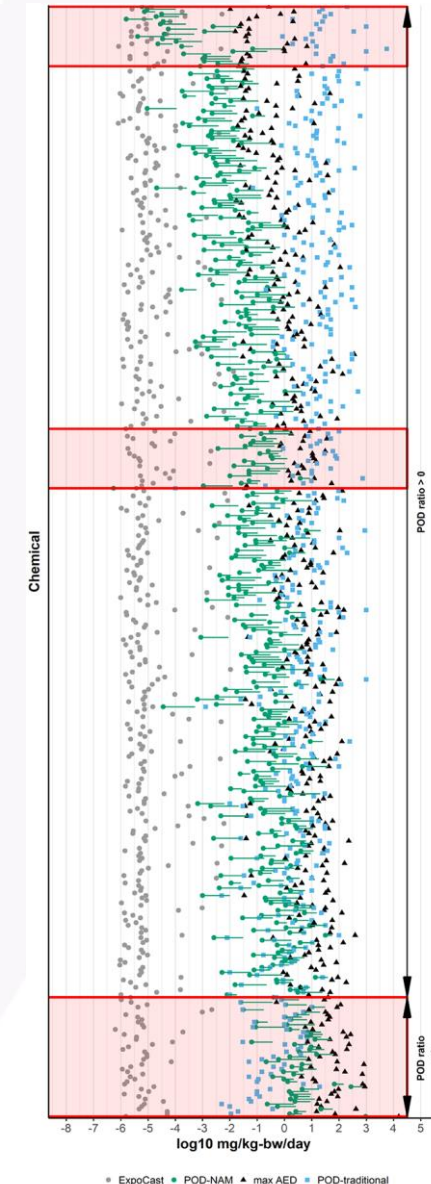
### Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization

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



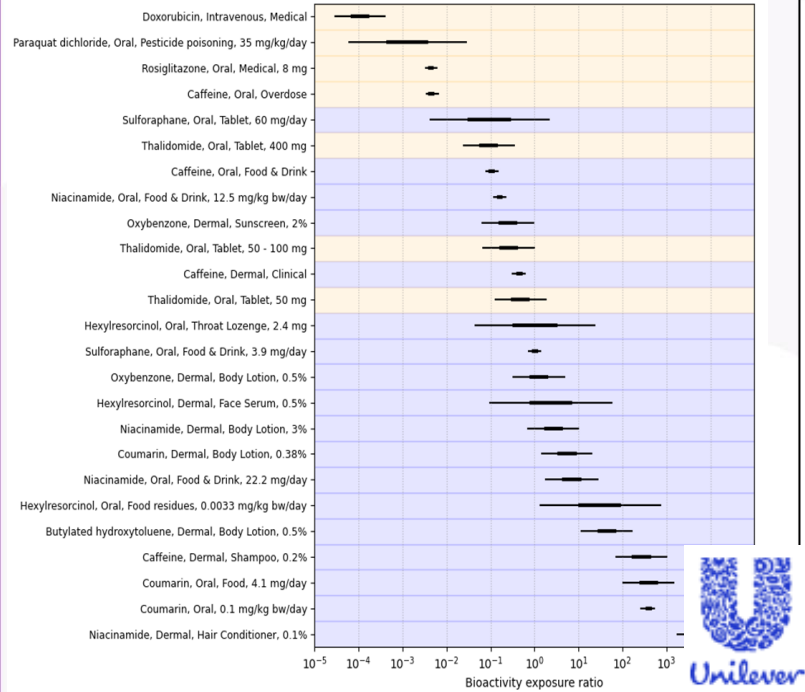
Of the 448 substances, 89% had a  $POD_{NAM,95}$  that was less than the traditional  $POD$  ( $POD_{traditional}$ ) value.

Bioactivity:exposure ratios (BERs), useful for identification of priority substances, demonstrated that high-throughput exposure predictions were greater than the  $POD_{NAM,95}$  for 11 substances.



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

>85 scenarios  
Pilot + Full study



Benchmark BER against risk category for each exposure scenario

46 compounds

Science Approach Document

Bioactivity Exposure Ratio:  
Application in Priority Setting and Risk Assessment

Health Canada

March 2021

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

30 compounds

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 Testi No. 349

>22 compounds

EU-ToxRisk  
An Integrated European 'Flagship' Program  
Driving Mechanism-based Toxicity Testing and Risk Assessment for the 21<sup>st</sup> Century

Case Study 16 Reporting Template

Team: 2  
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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 tier of exposure for risk assessment
- Can NAM be used to assess the potential for systemic toxicity based on pre-determined exposure scenarios based on typical use of the chemical for the intended use of the chemical?
- Identify adverse exposures within the risk assessment such that they can be further investigated
- Generate the cases to which the use of NAM can be identified, justified in place of required test or proposed test for regulatory testing.

Pragmatic Objectives

- Provide risk assessment experience of experts in diverse roles, many of whom are not currently conducting risk assessments for their day to day work
- Assess the applicability of NAM to assess the potential for systemic toxicity
- Generate testing or regulatory testing to make complete risk assessment decisions

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



# Summary

- Exposure-led approach to determine protection through a BER (MoS)
- Focus on weight of evidence to show tools can be integrated to make a safety decision - requires diverse expertise
- Strength derived from a combination of targeted and broad unbiased tools – hypothesis led
- NAMs not standard - need to ensure robustness/quality of tools and include estimations of uncertainty to aid acceptance
- Utilise NAMs for further targeted follow where required to refine uncertainty e.g. metabolism
- Further evaluation, additional case studies internal/ in collaboration eg EPA, CosEU, EU-ToxRisk – as well as APCRA
- Dissemination required to progress assessment and build out confidence for broader stakeholder community on applicability domains/ remaining gaps



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