Next Generation Risk Assessment (NGRA): A case study approach

Carl Westmoreland
Maria Baltazar, Paul Carmichael
Safety & Environmental Assurance Centre, Unilever

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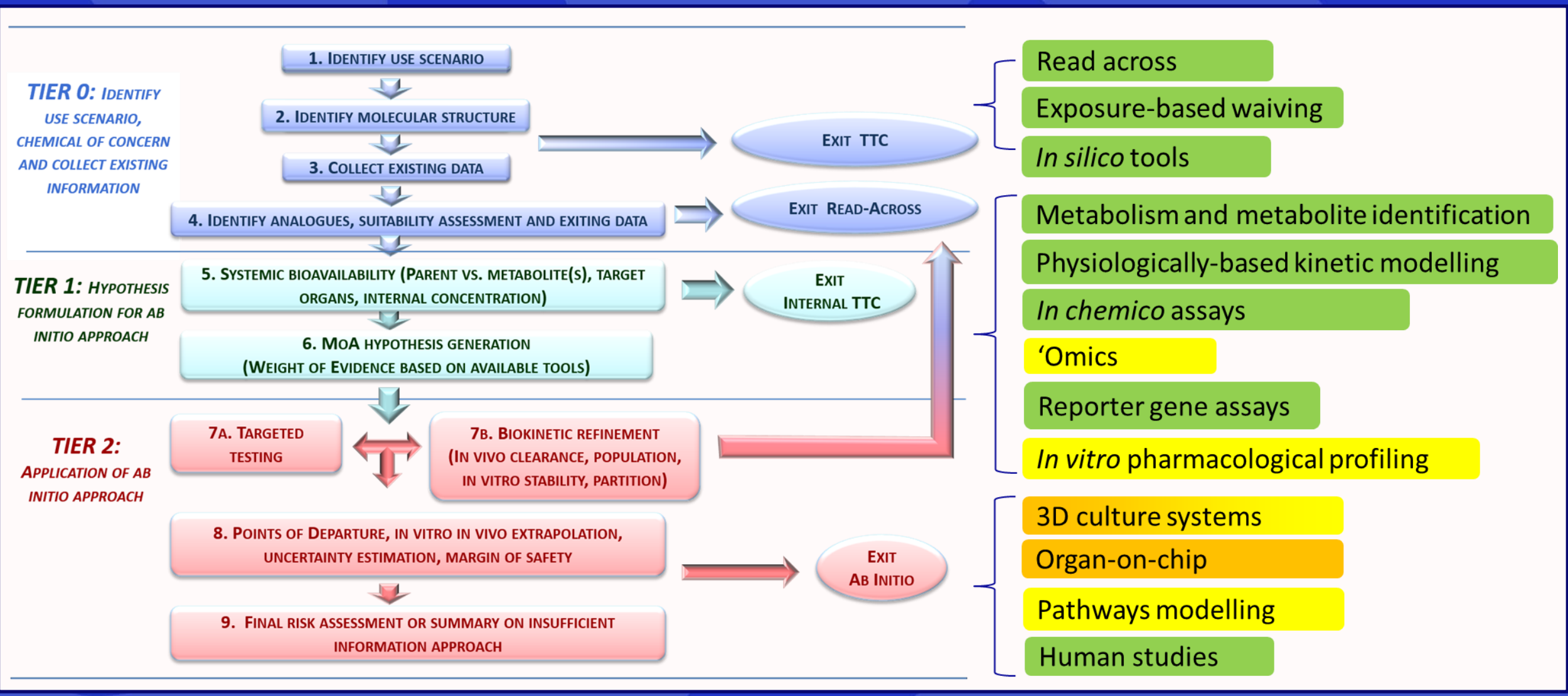


Computational Toxicology (2017) **4**, 31-44

Principles of NGRA from ICCR
Non-animal approaches in Cosmetic Risk Assessment

4 Main overriding principles:

- The overall goal is a human safety risk assessment
- The assessment is exposure led
- The assessment is hypothesis driven
- The assessment is designed to prevent harm

3 Principles describe how a NGRA should be conducted:

- Following an appropriate appraisal of existing information
- Using a tiered and iterative approach
- Using robust and relevant methods and strategies

2 Principles for documenting NGRA:

- Sources of uncertainty should be characterized and documented
- The logic of the approach should be transparently and documented

Computational Toxicology (2018) **7**, 20-26

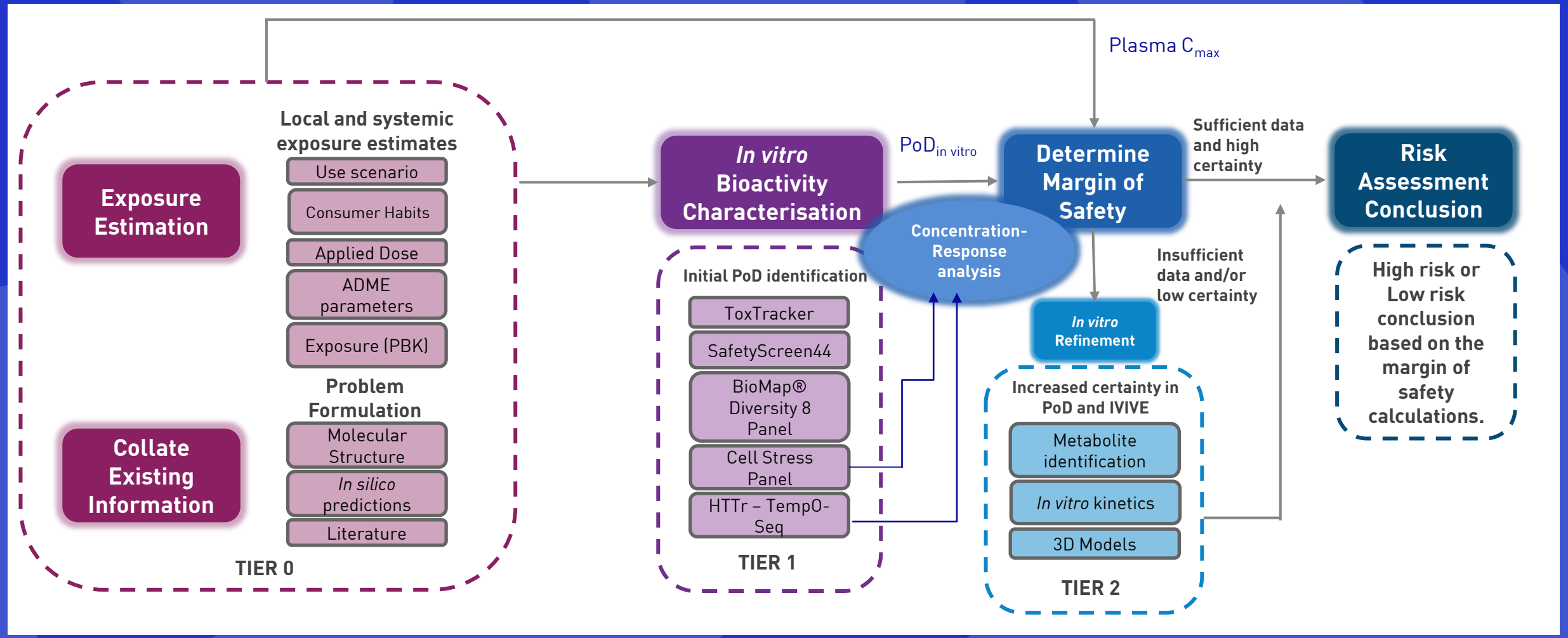
Case Study Approach… Imagine we have no data for: Coumarin

Safety assessment required for **0.1% coumarin in Face Cream**

Safety assessment required for **0.1% coumarin in Body lotion**

Baltazar *et al* (2020) Toxicological Sciences, Accepted

Ab Initio NGRA Framework

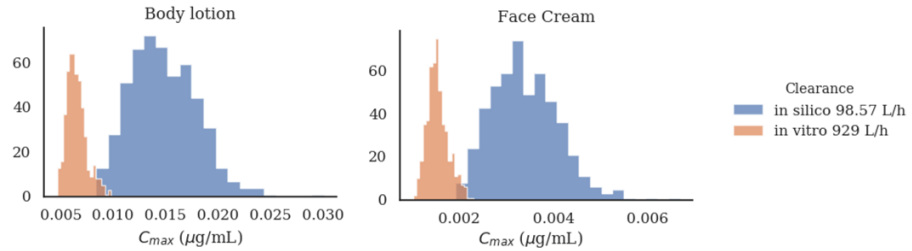


Baltazar *et al* (2020) Toxicological Sciences, Accepted

Systemic Bioavailability using PBK Modelling

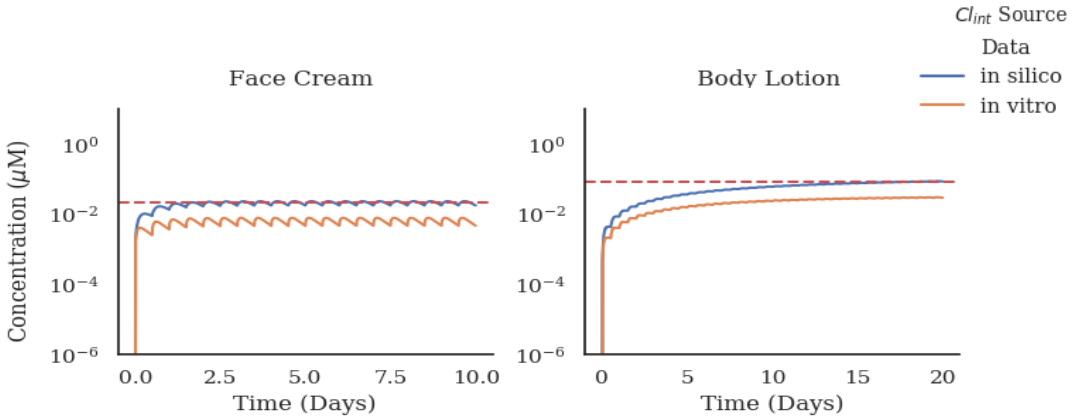
Key output parameters from uncertainty analysis:

Total Plasma C_{max} (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
Body lotion	0.01	0.01	0.018	0.019	0.02	0.022



Uncertainty & Population Variability

0.1% Face cream & body lotion in Europe

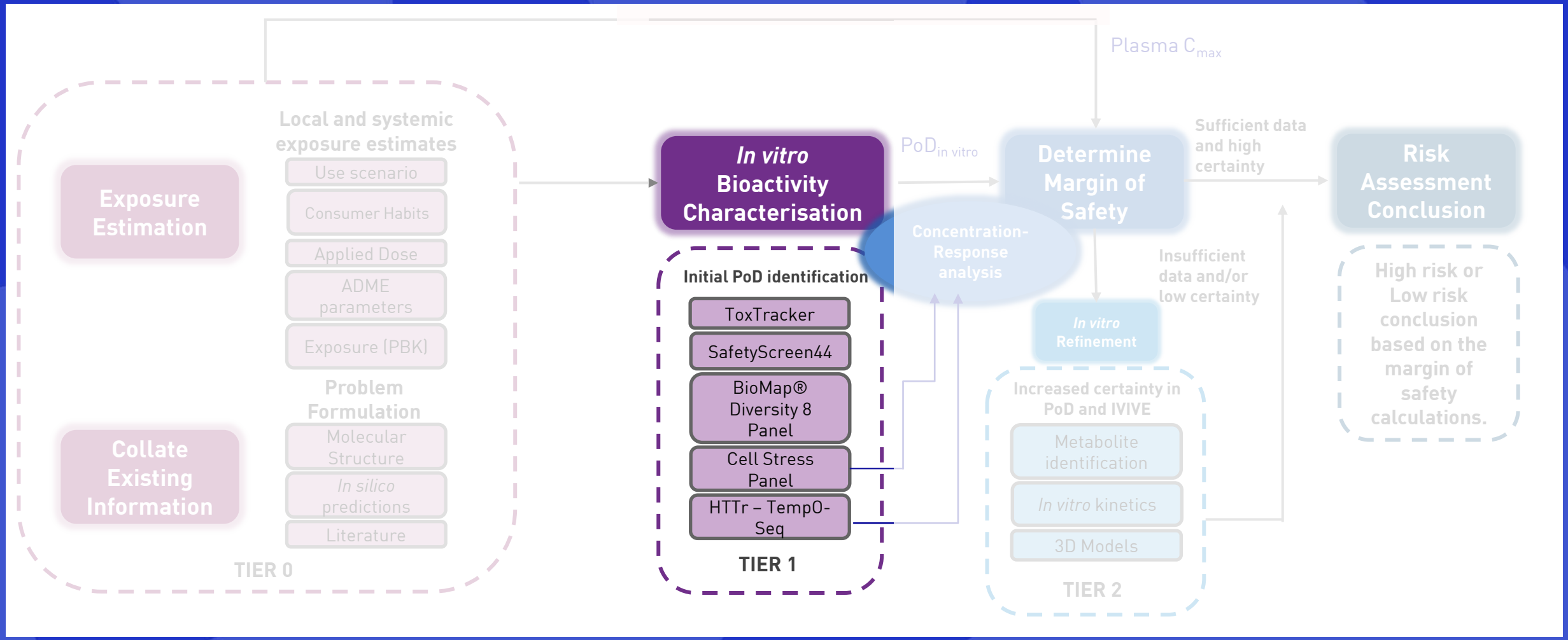


Physiologically-based kinetic modelling using GastroPlus® v9.5.

Estimations based on experimental data (Clint, fup, bpr, solubility, LogP). Skin penetration parameters were fitted against skin penetration data.

Moxon *et al* (2020) Toxicology in Vitro, **63** 104746

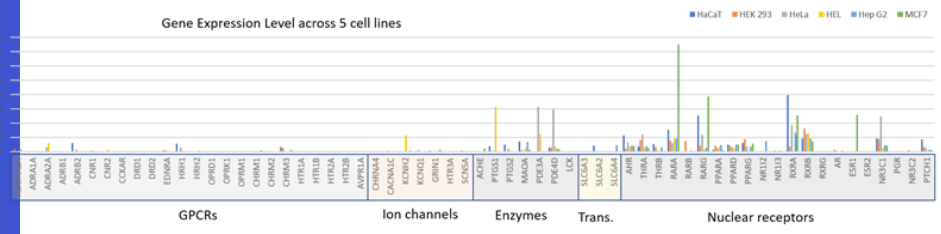
Ab initio NGRA Framework

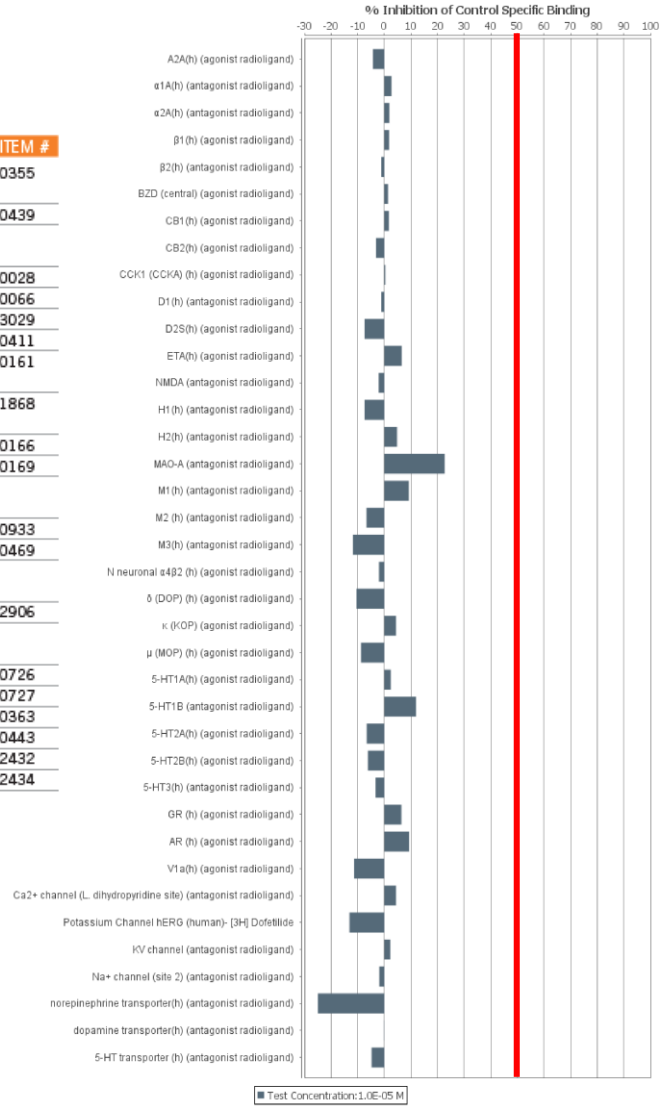


In vitro Bioactivity: Safety Screen

Bowes et al 2012. Nature Reviews: Drug Discovery 11 909-922

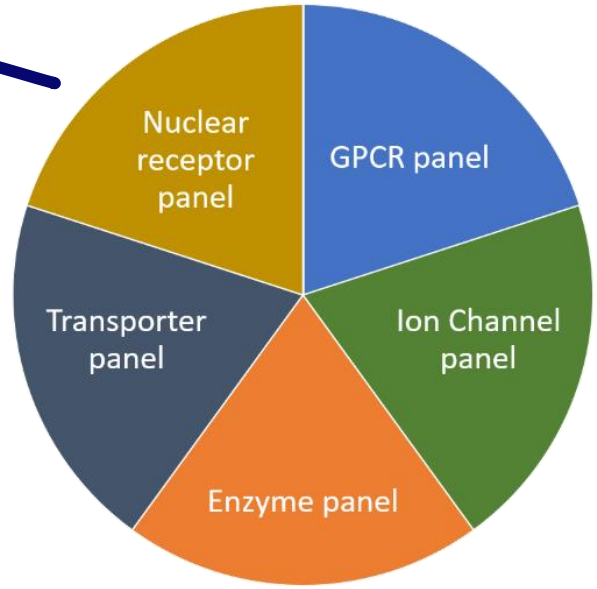
FAMILY	ASSAY	FORMAT	ITEM #
GPCR			
ADENOSINE	A_{3A}	•	0004
ADRENERGIC	$alpha_{1A}$		2338
	$alpha_{2A}$		0013
	$beta_1$	•	0018
	$beta_2$	•	0020
CANNABINOID	CB_1	•	0036
	CB_2	•	0037
CHOLECYSTOKININ	CCK_1 (CCK_A)	•	0039
DOPAMINE	D_1		0044
	D_{2L}	•	1322
ENDOTHELIN	ET_A	•	0054
HISTAMINE	H_1		0870
	H_2		1208
MUSCARINIC	M_1		0091
	M_2		0093
	M_3		0095
OPIOID & OPIOID-LIKE	$delta_2$ (DOP)	•	0114
	kappa (KOP)	•	1971
	mu (MOP)	•	0118
SEROTONIN	$5\text{-}HT_{1A}$	•	0131
	$5\text{-}HT_{1B}$		0132
	$5\text{-}HT_{2A}$	•	0471
	$5\text{-}HT_{2B}$	•	1333
VASOPRESSIN	V_{1a}	•	0159
TRANSPORTERS			
DOPAMINE	dopamine transporter		0052
NOREPINEPHRINE	norepinephrine transporter		0355
SEROTONIN	5-HT transporter		0439
ION CHANNELS			
GABA CHANNELS	BZD (central)	•	0028
GLUTAMATE CHANNELS	NMDA		0066
NICOTINIC CHANNELS	N neuronal α4β2	•	3029
SEROTONIN CHANNELS	$5\text{-}HT_3$		0411
Ca^{2+} CHANNELS	Ca^{2+} channel (L, dihydropyridine site)		0161
K^+ CHANNELS	hERG (membrane preparation)		1868
	K_v channel		0166
Na^+ CHANNELS	Na^+ channel (site 2)		0169
NUCLEAR RECEPTORS			
STEROID NUCLEAR RECEPTORS	AR	•	0933
	GR	•	0469
KINASES			
CTK	Lck kinase		2906
OTHER NON-KINASE ENZYMES			
AA METABOLISM	COX_1		0726
	COX_2		0727
MONOAMINE & NEUROTRANSMITTER	acetylcholinesterase		0363
	MAO-A		0443
PHOSPHODIESTERASES	PDE3A		2432
	PDE4D2		2434





All binding and enzymatic assay results were negative at 10 uM, including COX-1 and COX-2

No receptor/target-led pharmacological effect



SafetyScreen44™ Panel

Immunomodulatory Bioactivity: BioMap® Diversity 8 Panel

BioMAP systems contain human primary cell types (or combinations) that are stimulated to replicate complex cell and pathway interactions of vascular inflammation, immune activation and tissue remodelling

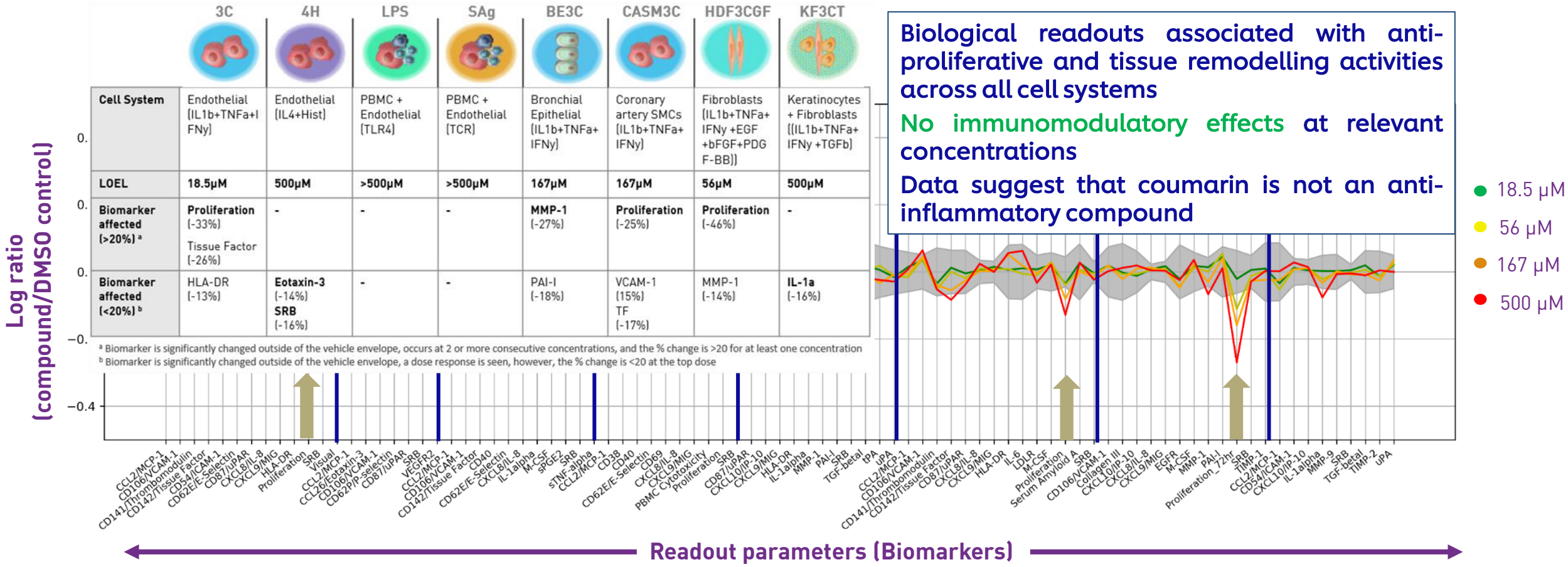
	3C	4H	LPS	SAg	BE3C	CASM3C	HDF3CGF	KF3CT
Cell System	Endothelial (IL1b+TNFa+IFNy)	Endothelial (IL4+Hist)	PBMC + Endothelial (TLR4)	PBMC + Endothelial (TCR)	Bronchial Epithelial (IL1b+TNFa+IFNy)	Coronary artery SMCs (IL1b+TNFa+IFNy)	Fibroblasts (IL1b+TNFa+IFNy +EGF +bFGF+PDGF-BB)]	Keratinocytes + Fibroblasts ([IL1b+TNFa+IFNy +TGFb)
LOEL	**18.5µM**	**500µM**	**>500µM**	**>500µM**	**167µM**	**167µM**	**56µM**	**500µM**
Biomarker affected (>20%) [a]	**Proliferation** (-33%) Tissue Factor (-26%)	-	-	-	**MMP-1** (-27%)	**Proliferation** (-25%)	**Proliferation** (-46%)	-
Biomarker affected (<20%) [b]	HLA-DR (-13%)	**Eotaxin-3** (-14%) **SRB** (-16%)	-	-	PAI-I (-18%)	VCAM-1 (15%) TF (-17%)	MMP-1 (-14%)	**IL-1a** (-16%)

[a] Biomarker is significantly changed outside of the vehicle envelope, occurs at 2 or more consecutive concentrations, and the % change is >20 for at least one concentration
[b] Biomarker is significantly changed outside of the vehicle envelope, a dose response is seen, however, the % change is <20 at the top dose

Biological readouts associated with anti-proliferative and tissue remodelling activities across all cell systems

No immunomodulatory effects at relevant concentrations

Data suggest that coumarin is not an anti-inflammatory compound



In Vitro Bioactivity: Cell Stress Panel

~40 Biomarkers; 3 Timepoints; 8 Concentrations; ~10 Stress Pathways

Step 1

Selection of stress pathways

Mitochondrial Toxicity , Oxidative Stress, DNA damage, Inflammation, ER Stress, Metal Stress, Heat Shock, Hypoxia, Cell Health

Selection of biomarkers, probes or antibodies and optimisation of high-content imaging

Selection of cell line, exposure scenario and timepoints

HepG2 cell line, single exposure, 1h, 6h and 24h

Step 2

Selection of chemicals according to different classes and exposure scenarios (based on typical use of compound)

Non-stress inducers
- Caffeine (beverages, cosmetics)
- Coumarin (food, cosmetics)
- Niacinamide (food, cosmetics)
- Phenoxyethanol (cosmetics)

Stress inducers
- CDDO-Me (drug)
- Sulforaphane (food)
- DEM (industrial chemical)
- tBHQ (antioxidant)
- Doxorubicin (drug)
- Diclofenac (drug)
- Triclosan (antimicrobial)
- Troglitazone (drug)
- Pioglitazone (drug)
- Rosiglitazone (drug)

Step 3

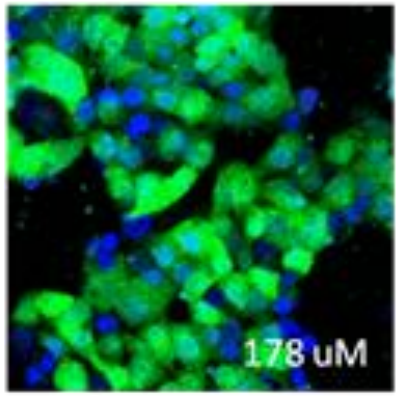
Selection of *in vitro* concentrations based upon realistic human exposures

Information on human exposure obtained from human clinical trials or PBK modelling

Selection of 8 *in vitro* concentrations (upper bound limited by ~20% cytotoxicity

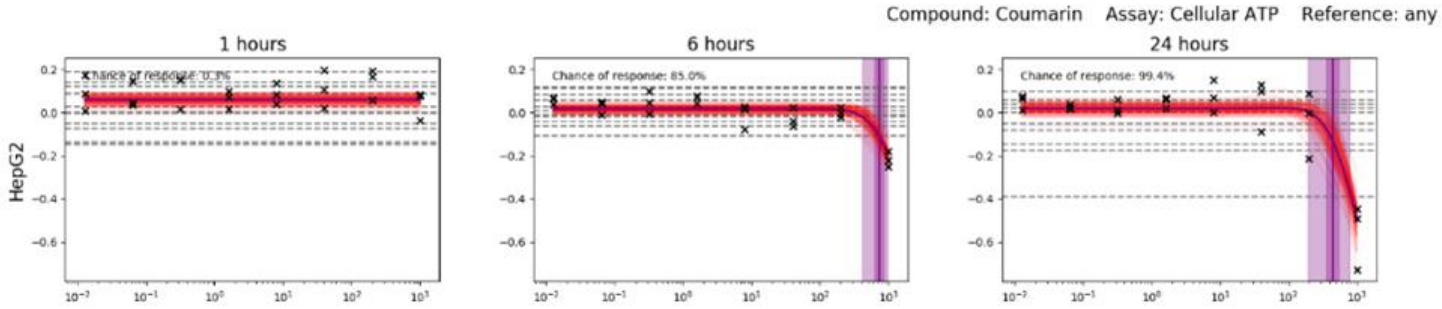
Key
- Exposure scenario adopted for chemical is 'high risk' (from consumer goods perspective).
- Exposure scenario adopted for chemical is 'low risk' (from consumer goods perspective).

- Mitochondrial Toxicity
- Oxidative Stress
- DNA damage
- Inflammation
- ER Stress
- Metal Stress
- Osmotic Stress
- Heat Shock
- Hypoxia
- Cell Health



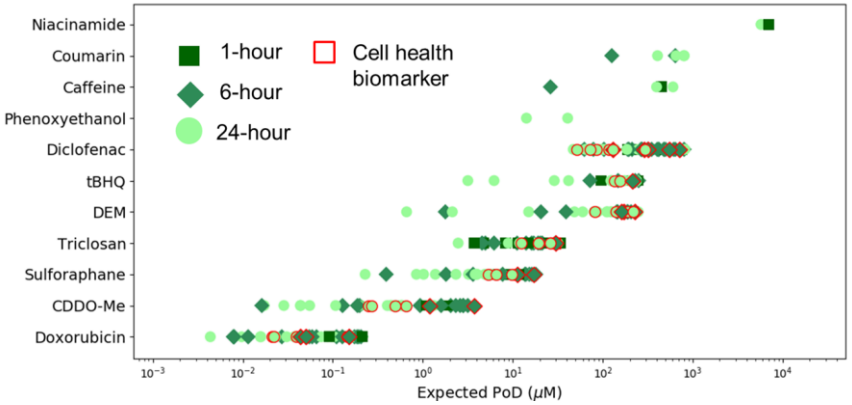
Hatherell *et al* (2020) Toxicological Sciences, Accepted

In Vitro Bioactivity: Cell Stress Panel



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

Summary with PoD for cell stress biomarkers:



- Coumarin not very active in comparison to known 'high risk compounds' like doxorubicin, diclofenac etc.
- Cell count, cellular ATP, GSH, IL-8, Phospholipids, OCR, reserve capacity and steatosis showed a dose response

In Vitro Bioactivity: Tempo-Seq Technology

High-Throughput Transcriptomics Gene Expression Profiling (HTTr)

Defining a safe operating exposure for systemic toxicity using a **NOTEL** (No Transcriptional Effect Level)

NOTEL is the derived concentration of a compound that does not elicit a meaningful change in gene expression (i.e. the threshold of the concentration that elicits minimal mechanistic activity)

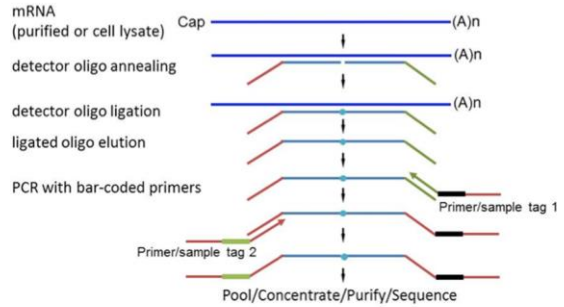
Cell lines (chosen to express a range of relevant receptors)

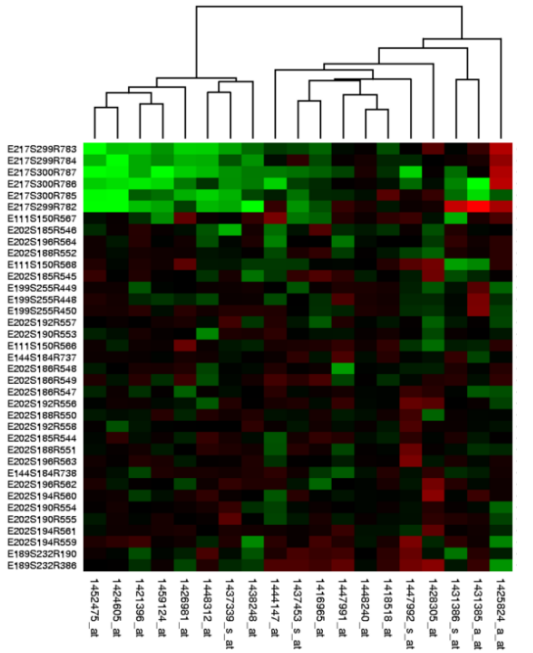
MCF-7 – human breast adenocarcinoma cell line

HepG2 – human liver carcinoma

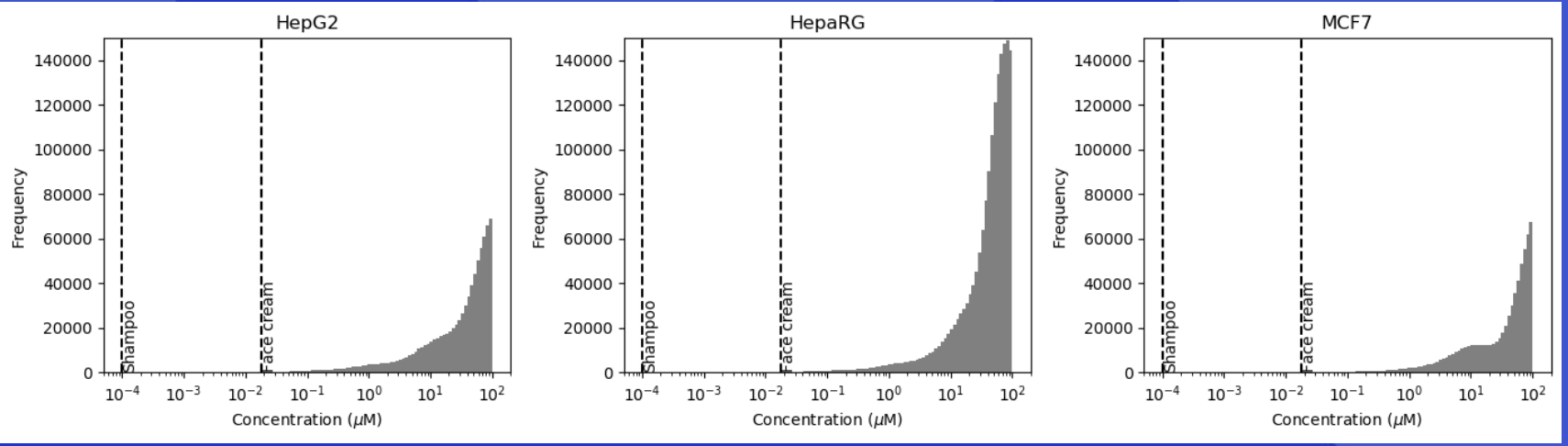
HepaRG – terminally differentiated hepatic cells that retain many characteristics of primary human hepatocytes + as spheroids

N-HEK – primary normal human epidermal keratinocytes





In Vitro Bioactivity: Tempo-Seq Technology



- Coumarin dose range 0.001uM to 100uM
- 24 hour time point
- QC and normalisation in DESeq2
- BMDExpress2 applied to determine NOTEL
  (3 pathway approaches)

Cell Model	HepG2	MCF7	HepaRG 2D
Pathway Level Tests	(308 pathways)	(0 pathways)	(17 pathways)
20 pathways with the lowest pvalue 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	(1570 genes)	(47 genes)	(87 genes)
Mean BMD of 20 genes with largest fold change	6	3	54
Mean BMD of Genes between 25th and 75th percentile	17	1	59

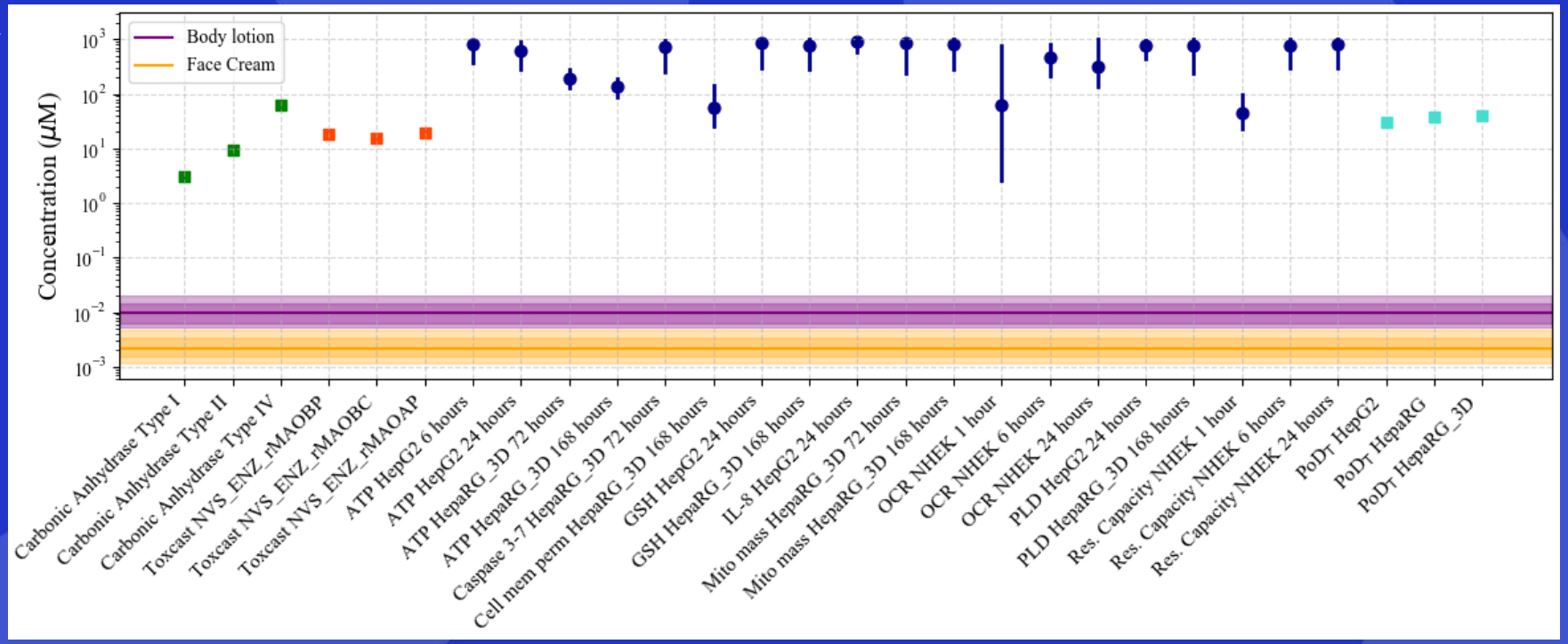
Margin of Safety considering PODs and Exposure

PoDs and plasma C_{max} (μM) are expressed as total concentration

C_{max} expressed as a distribution:

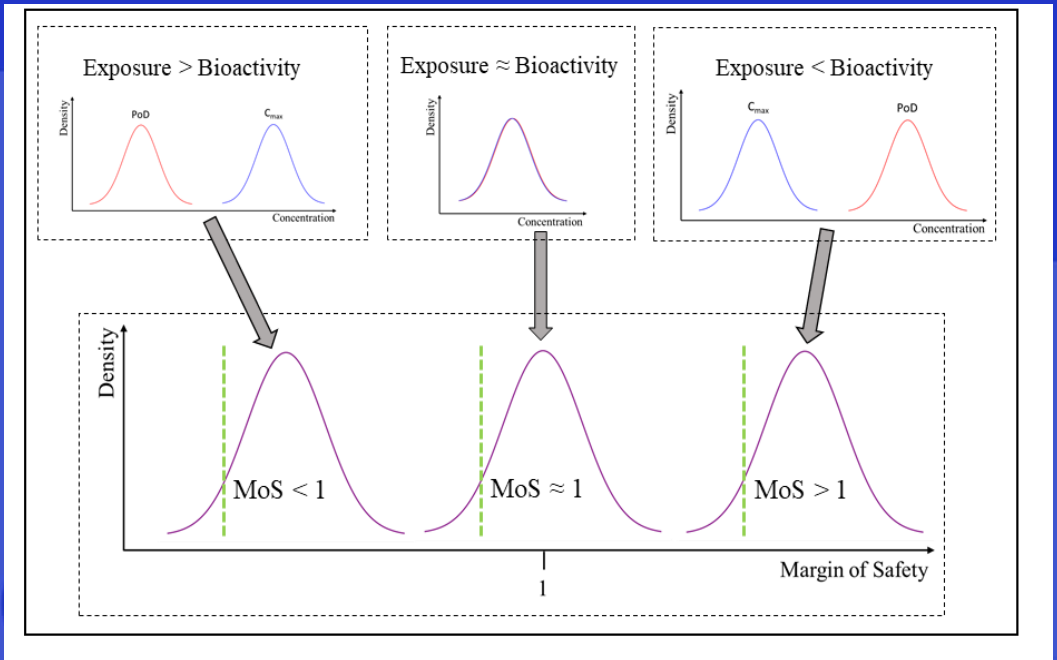
- Line = median (50th percentile)
- Inner band = 25th-75th percentile
- Outer band = 2.5th-97.5th percentile (95th credible interval)

PubChem | ToxCast | Cell Stress Panel | HTTr

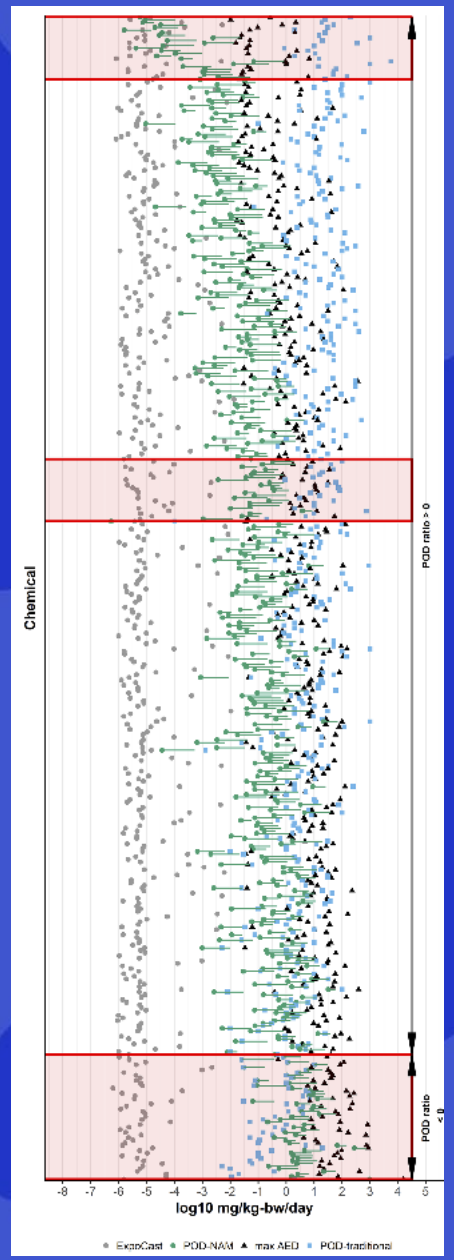


Application of *Ab Initio* Approach: Risk Assessment (NGRA)

Margin of safety is the **fold difference** between the **Cmax** and the ***in vitro*** **POD**



Technology	Cell line/ Enzyme/Biomarker	Face cream Min. 5th percentile MoS	Body Lotion Min. 5th percentile MoS
Cell stress panel	HepG2 (ATP, 24h)	96738	22048
Cell stress panel	NHEK (OCR 1h)	1330	**295**
HTTr	HepG2 (24h)	7223	1618
HTTr	HepaRG (24h)	8864	1986
Toxcast	MAO B	3711	831
PubChem	Carbonic Anhydrase Type I	**706**	**158**
PubChem	Carbonic Anhydrase Type II	2140	479
PubChem	Carbonic Anhydrase Type VI	14652	3282
Cell stress panel	HepaRG_3D (cell mem perm 168h)	9601	2197
HTTr	HepaRG_3D_24h	9538	2137



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

"The primary objective of this work was to compare PODs based on high-throughput predictions of bioactivity, exposure predictions, and traditional hazard information for 448 chemicals"

Comparison of the Exposure, POD_{NAM}, and $POD_{traditional}$. Comparison of ExpoCast (grey circles), POD_{NAM} (green circles), maximum AED (black triangles), and $POD_{traditional}$ values (blue boxes) for 448 substances. The green line segment indicates the $POD_{NAM, 95}$ to $POD_{NAM, 50}$.

Conclusions

Non-animal safety assessments for cosmetics are moving from '*might be possible in theory*' to '*case studies to evaluate*'

NGRA is a framework of non-standard, bespoke data-generation, driven by the risk assessment questions

- **Enabling a transition from using data from tests in live animals to one founded on understanding the effects of chemicals in humans using computational approaches and *in vitro* methods that evaluate changes in biologic processes using human cells**
- **Constructed from *in silico* modelling approaches and *in vitro* solutions**
- **Need to ensure quality/robustness of the non-standard (non-TG) work**
- **Importance of characterising uncertainty to allow informed decision-making**
- **Shortcomings will be addressed by current and future research**
- **More research, creativity and published examples needed to increase confidence for regulatory application.**

The approaches and challenges are not cosmetic-specific, how can different sectors learn together?

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