Exposure-based toxicity testing and translation into global requirements

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Unilever

Exposure-based toxicity testing

- Background
- Leading with Exposure
- Examples from the world of Cosmetics
- Translation into global requirements

Ensuring Safe Ingredients for Cosmetic Products

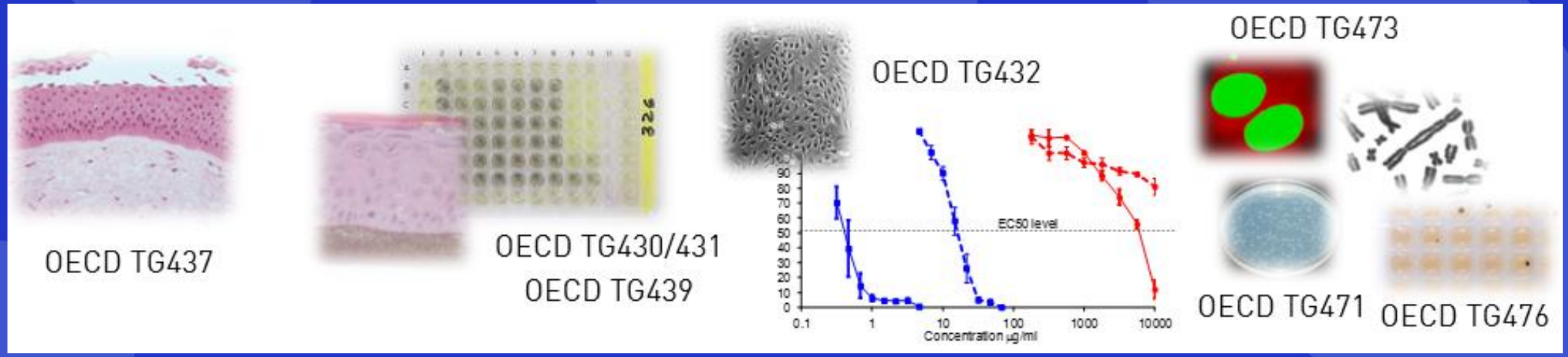
Risk Based Approach:

Considers both the hazard and the exposure to evaluate the risk

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

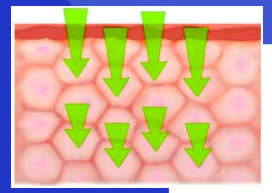
No pre-market authorization for most product types across the world – emphasis on manufacturer to show safe use

Use of Existing OECD *In Vitro* Approaches

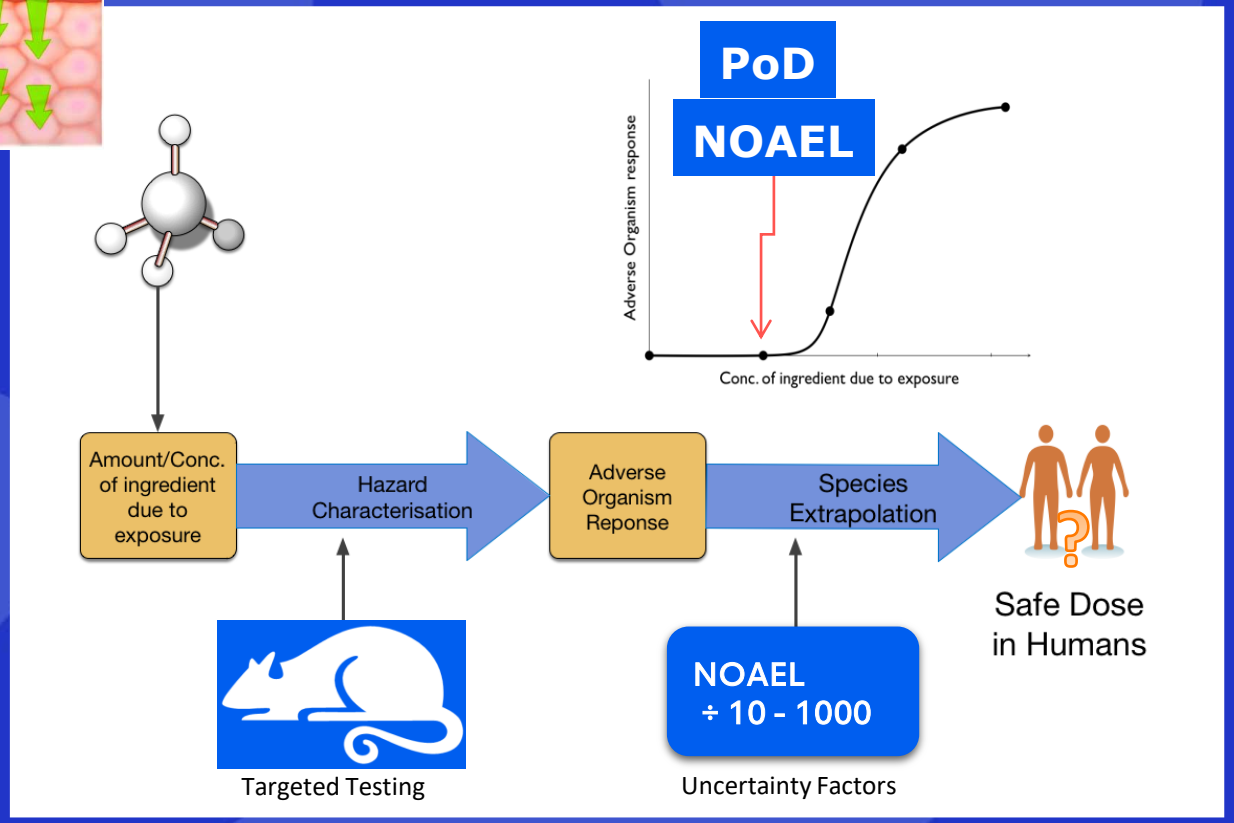


Skin and eye irritation; skin sensitization; phototoxicity; mutagenicity

What About Systemic Toxicity?



Is it safe?



~~e.g. 90 Day Repeat Dose Study~~

Existing approaches

Threshold of Toxicological Concern
(Yang et al 2017)
https://doi.org/10.1016/j.fct.2017.08.043

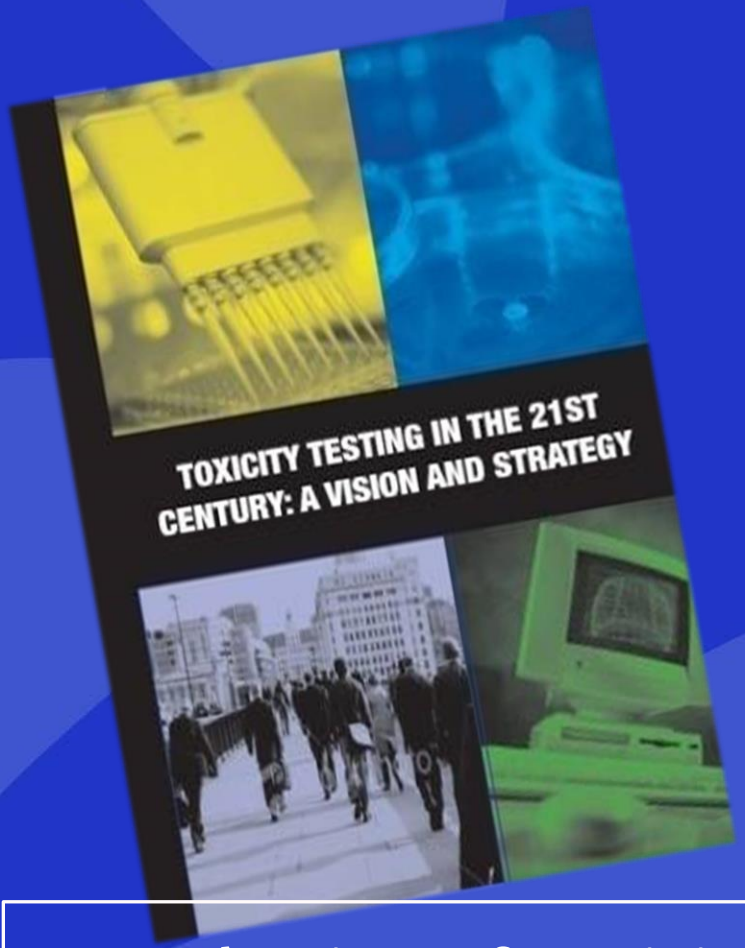
Read across

History of Safe Use
(Neely et al 2011)
https://doi.org/10.4103/0971-6580.85882

A new non-animal paradigm is needed...

...but replacement of animal test data is not the answer

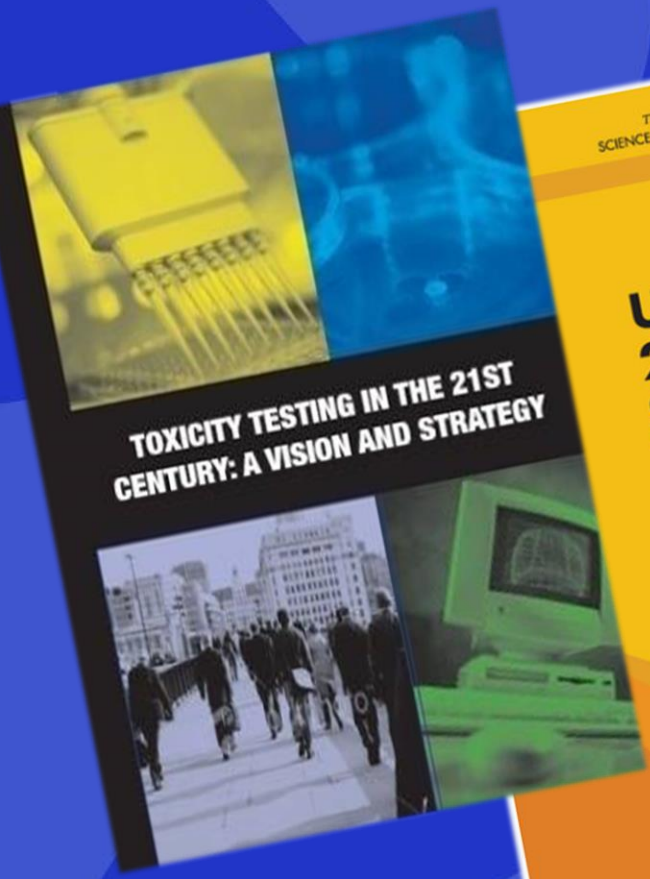
2007 Toxicity Testing in the 21st Century (TT21C)

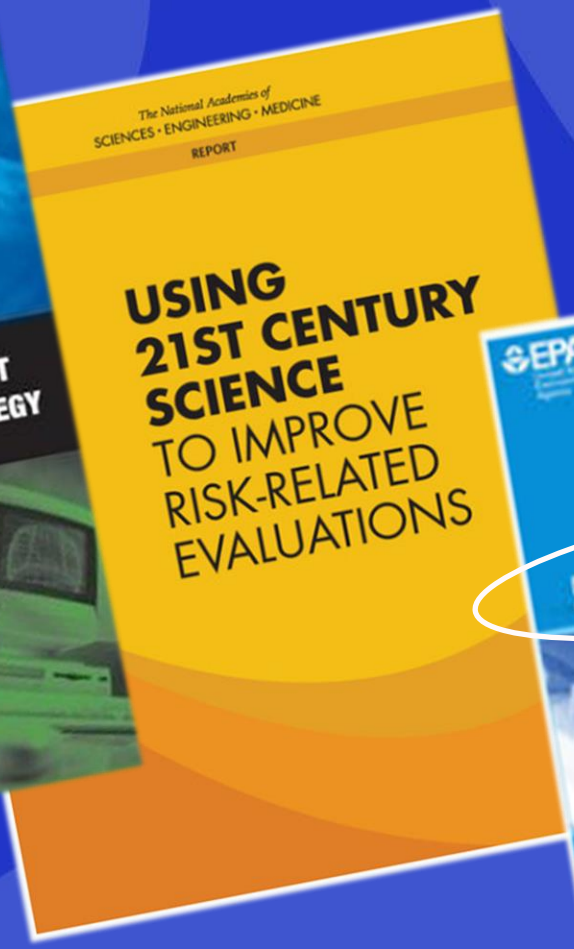


"Advances in toxicogenomics, bioinformatics, systems biology, and computational toxicology could transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin."

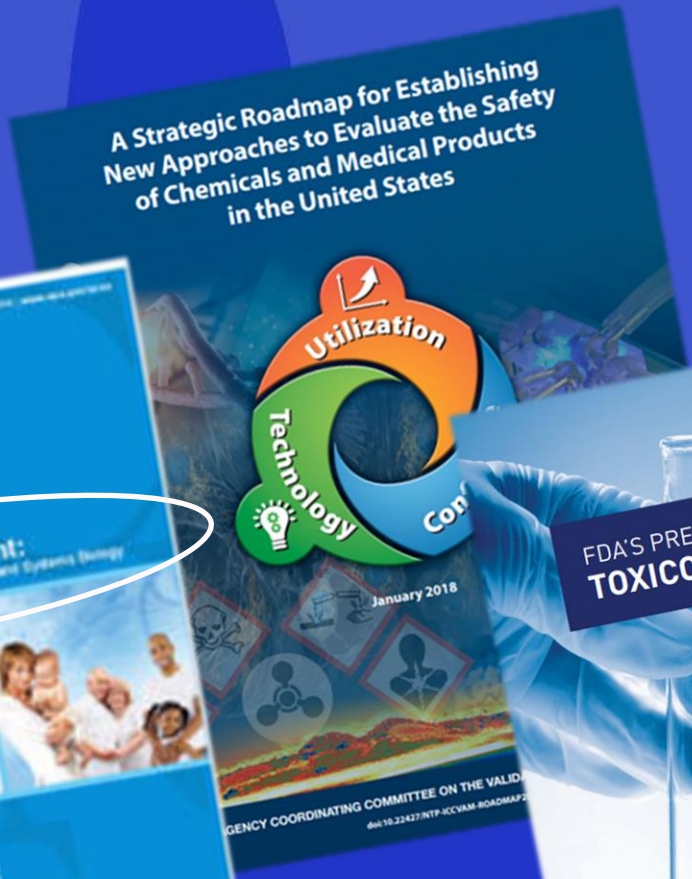
Perturbation of 'toxicity pathways' and stress responses

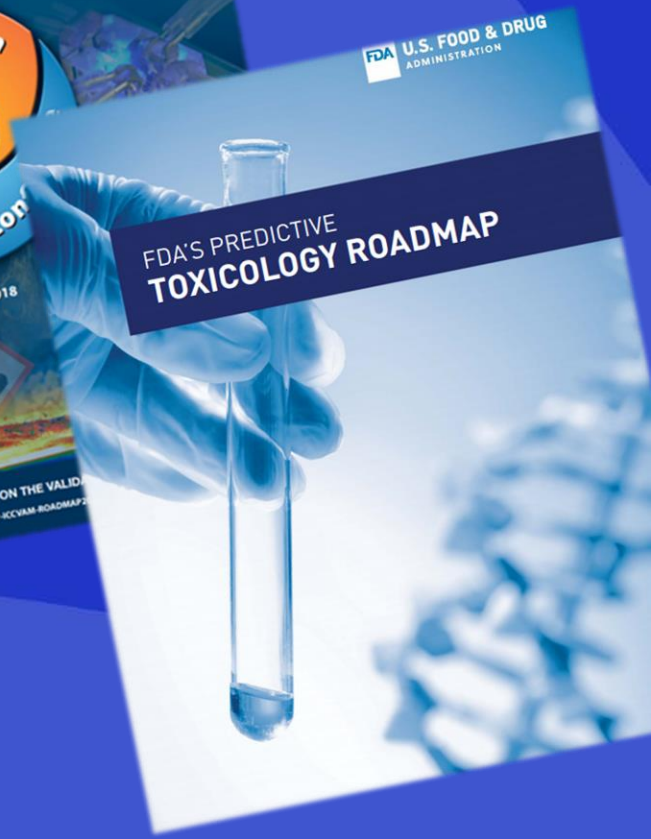
TT21C + NGRA


TOXICITY TESTING IN THE 21ST CENTURY: A VISION AND STRATEGY


The National Academies of
SCIENCES • ENGINEERING • MEDICINE
REPORT
USING 21ST CENTURY SCIENCE TO IMPROVE RISK-RELATED EVALUATIONS


EPA
Next Generation Risk Assessment:
Final Report


A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States
Utilization
Technology
January 2018


FDA U.S. FOOD & DRUG ADMINISTRATION
FDA'S PREDICTIVE
TOXICOLOGY ROADMAP

Principles of NGRA from ICCR

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:

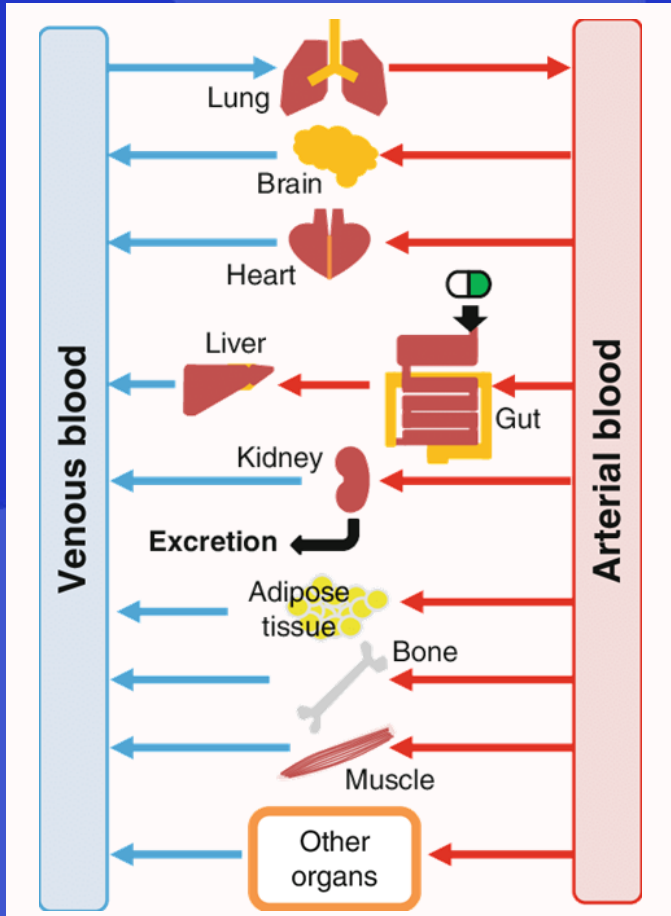
- Following an appropriate appraisal of existing information
- Using a tiered and iterative approach
- Using robust and relevant methods and strategies

2 Principles for documenting NGRA:

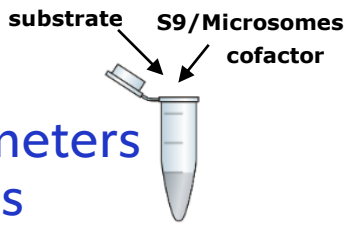
- Sources of uncertainty should be characterized and documented
- The logic of the approach should be transparently and documented

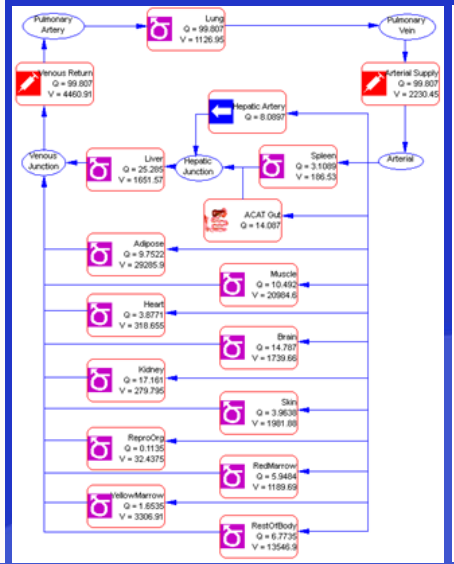
Dent *et al* ., (2018) *Comp Tox* 7:20-26

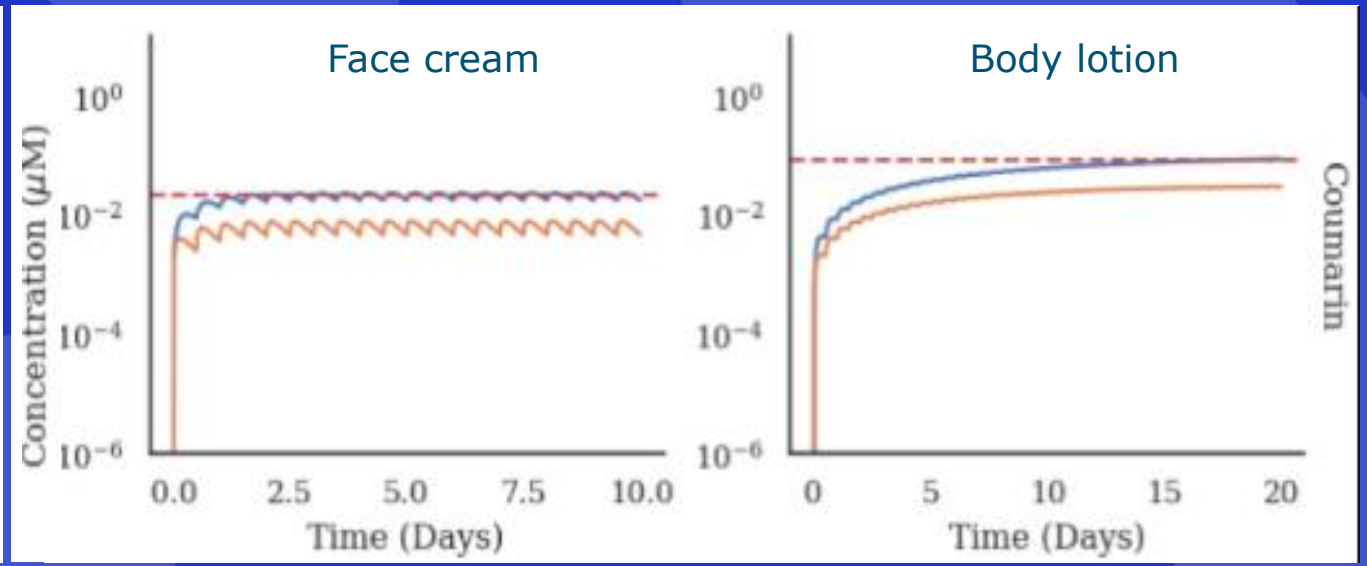
PBK (Physiologically Based Kinetic) Modelling



Model Input:
Physiological parameters
Partition coefficients
Kinetic constants (in vitro)

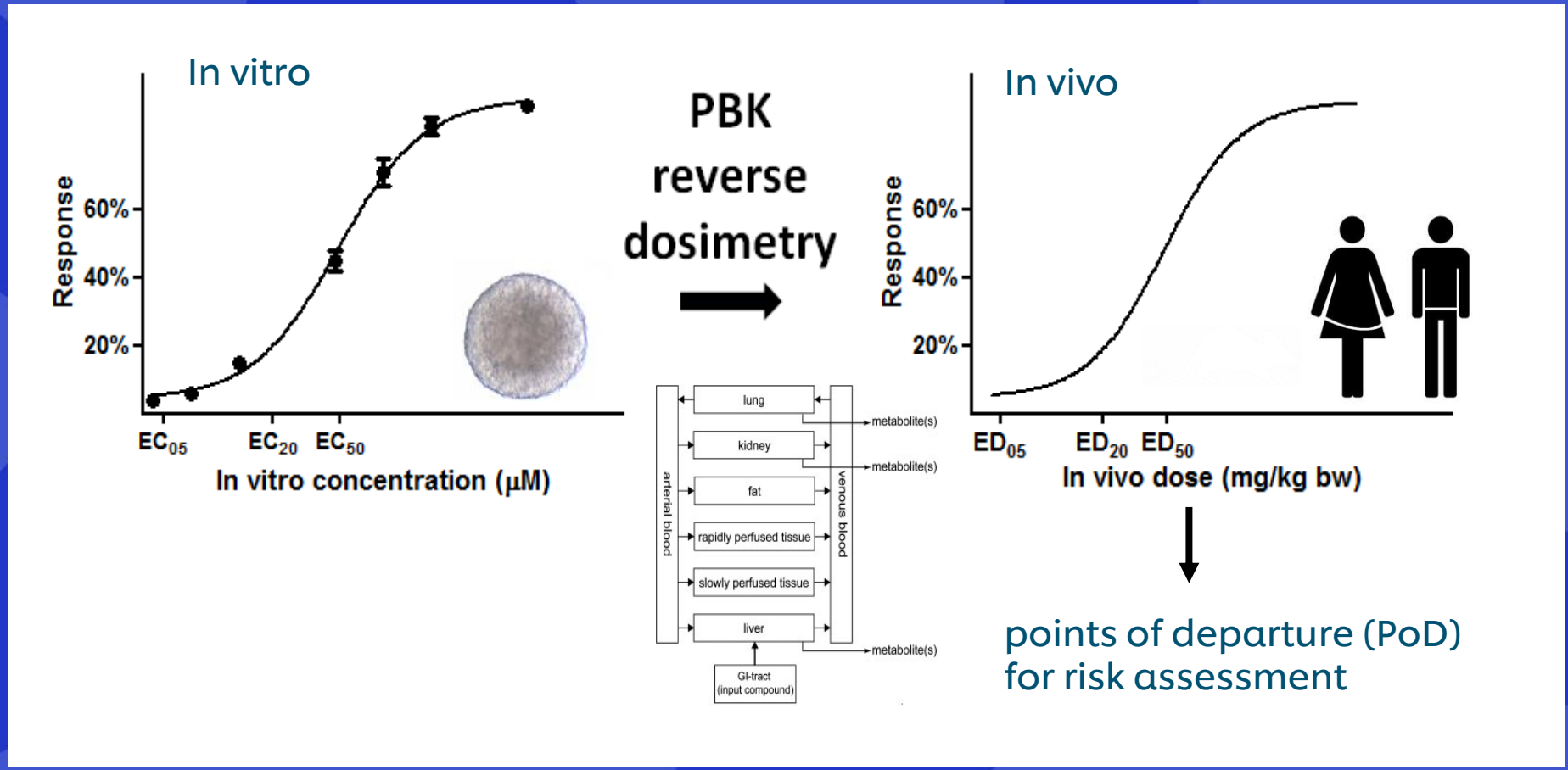






Moxon *et al.*, (2020) *TIV* 63
https://doi.org/10.1016/j.tiv.2019.104746

One Interpretation of TT21C: Quantitative *in vitro* to *in vivo* extrapolation



Another Interpretation: Tox21/ToxCast ~700 HTS Biological Pathways Assays

National Institute of Environmental Health Sciences (NIEHS) / National Toxicology Program (NTP)

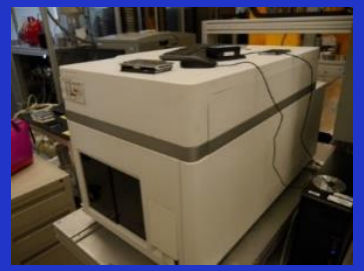
National Center for Advancing Translational Sciences (NCATS)

U.S. Food and Drug Administration (FDA)

National Center for Computational Toxicology (EPA)

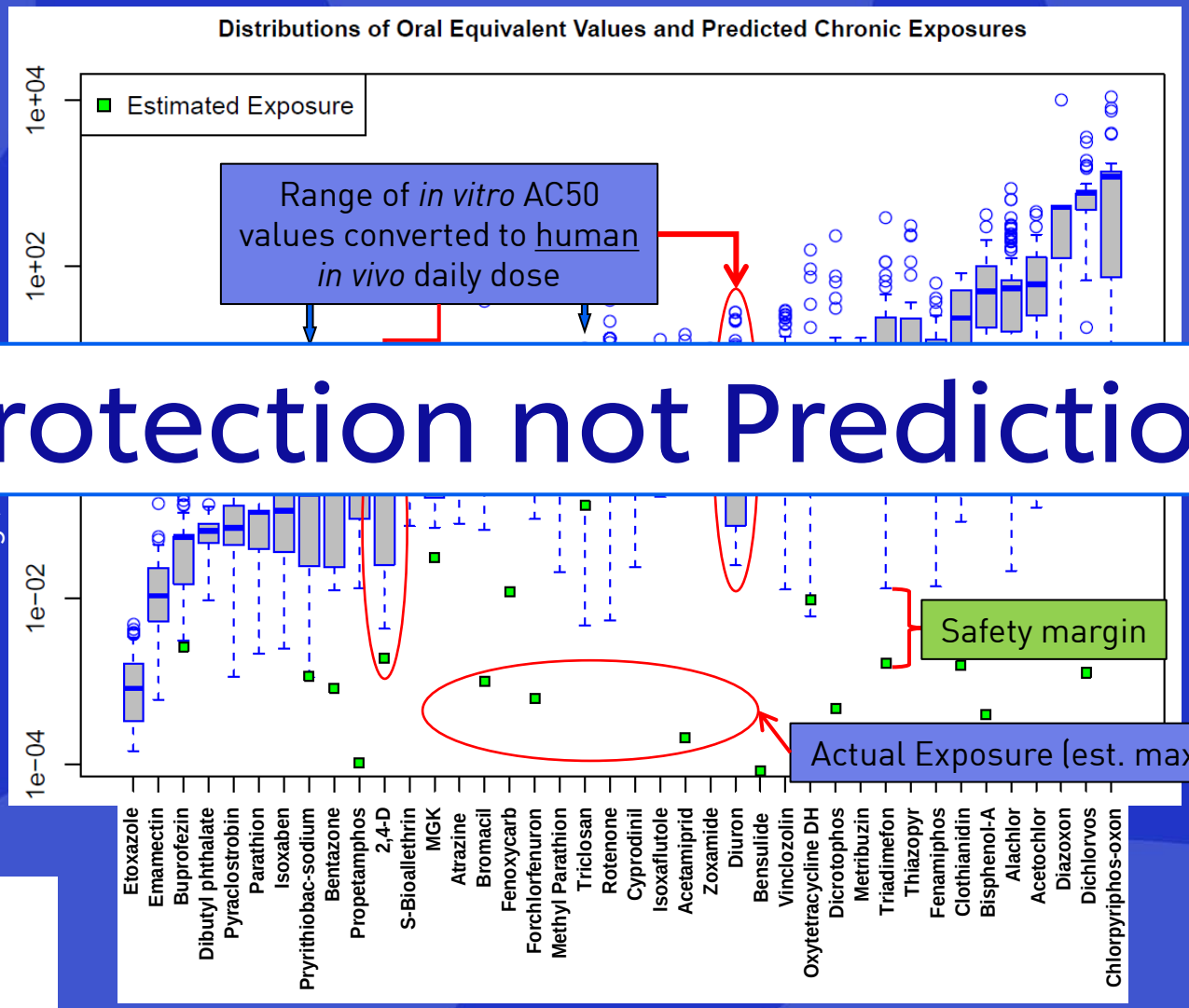





https://www.epa.gov/chemical-research/toxicity-forecasting

In Vitro Bioactivity vs Bioavailability



"Protection not Prediction"

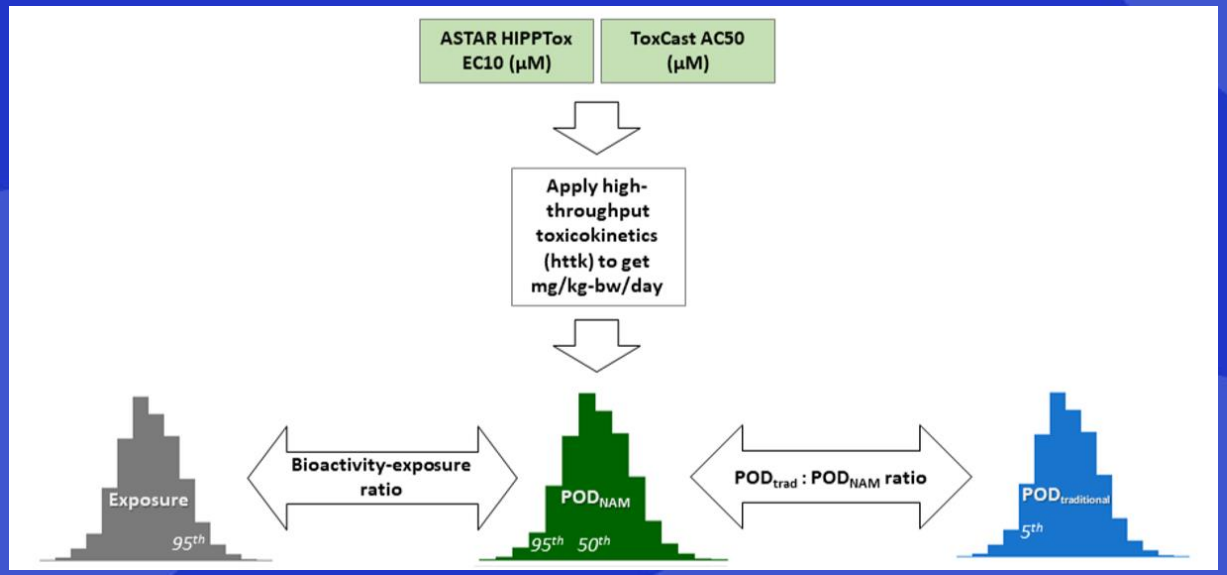
Hepatic clearance and plasma protein binding determinations

Slide from Dr Rusty Thomas, EPA, with thanks

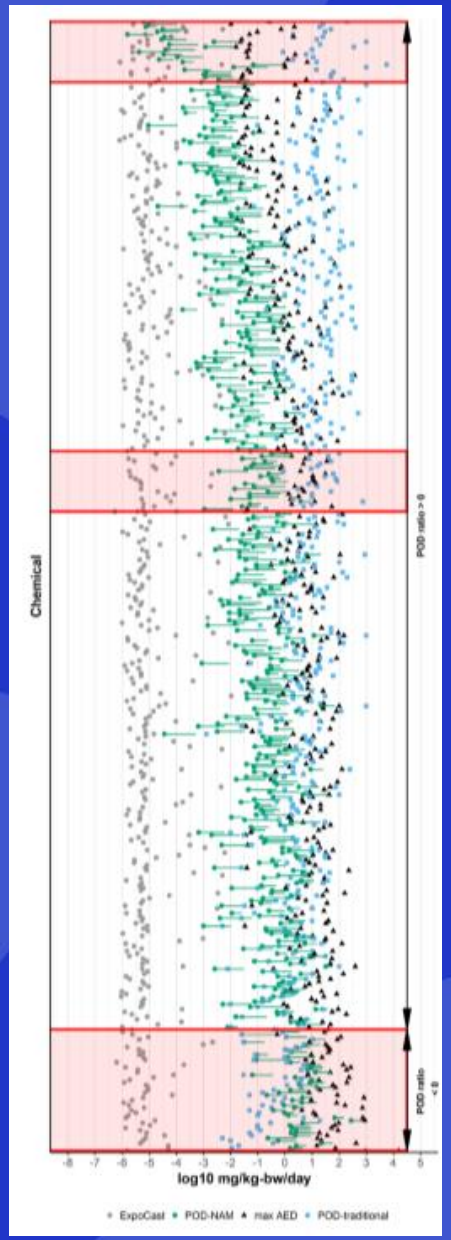
Rotroff, *et al.* Tox.Sci 2010 Vol 117/2 348-358

https://doi.org/10.1093/toxsci/kfq220

EPA, NTP, HC, A*STAR, ECHA, EFSA, JRC, RIVM…

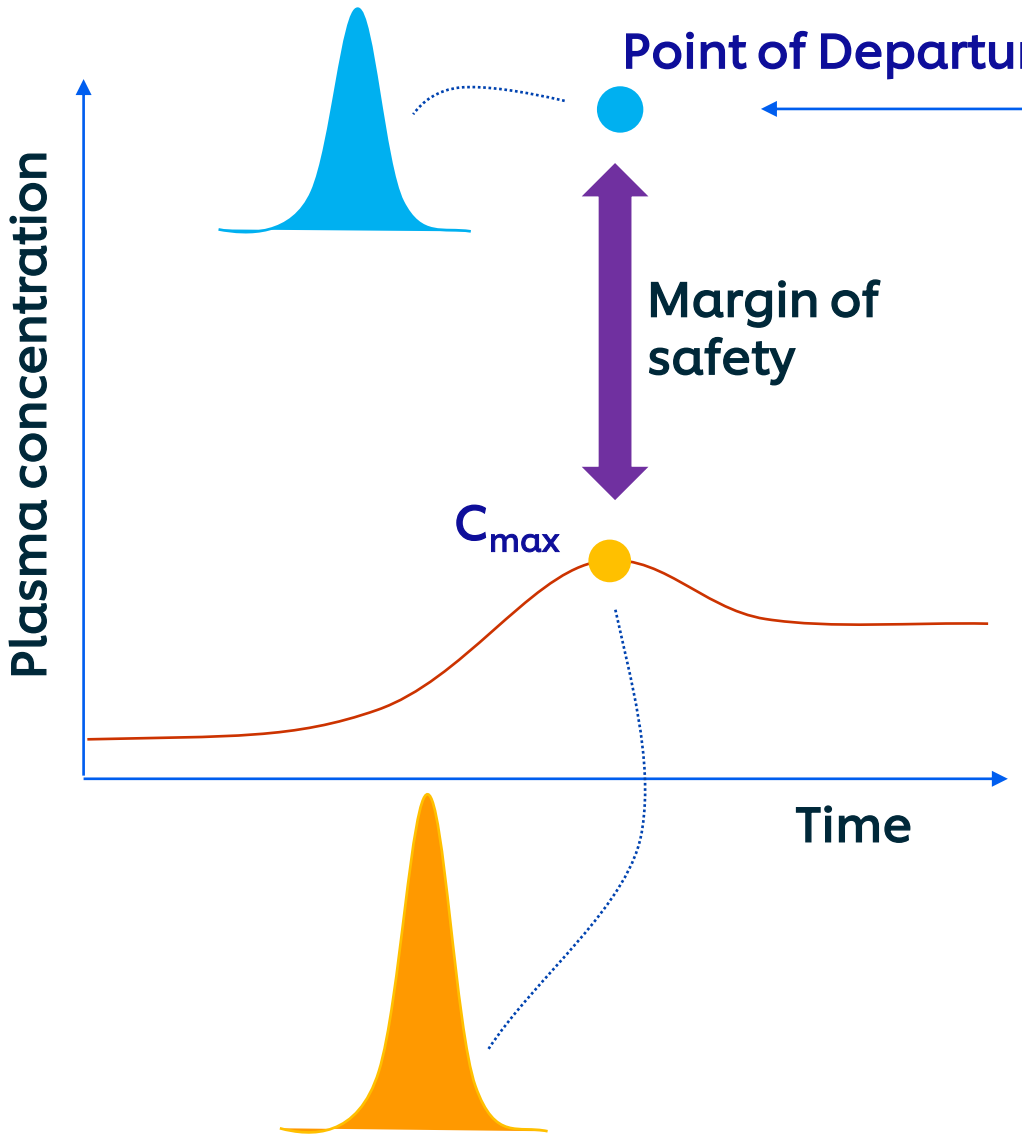


Katie Paul-Friedman *et al.* 2019 *Tox Sci* 173(1): 202-225

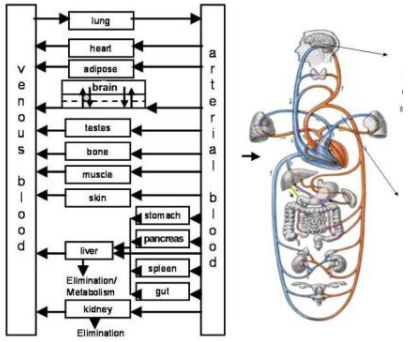


414/448 chemicals =
92% of the time this naïve approach appears conservative

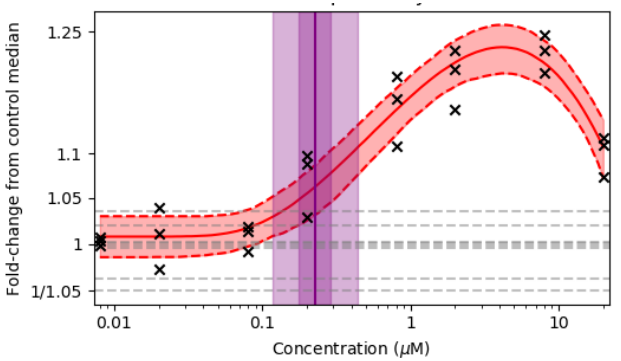
The Margin of Safety Approach



Exposure models (PBK, free/total concentration)



Point of departure derived from *in vitro* concentration-response



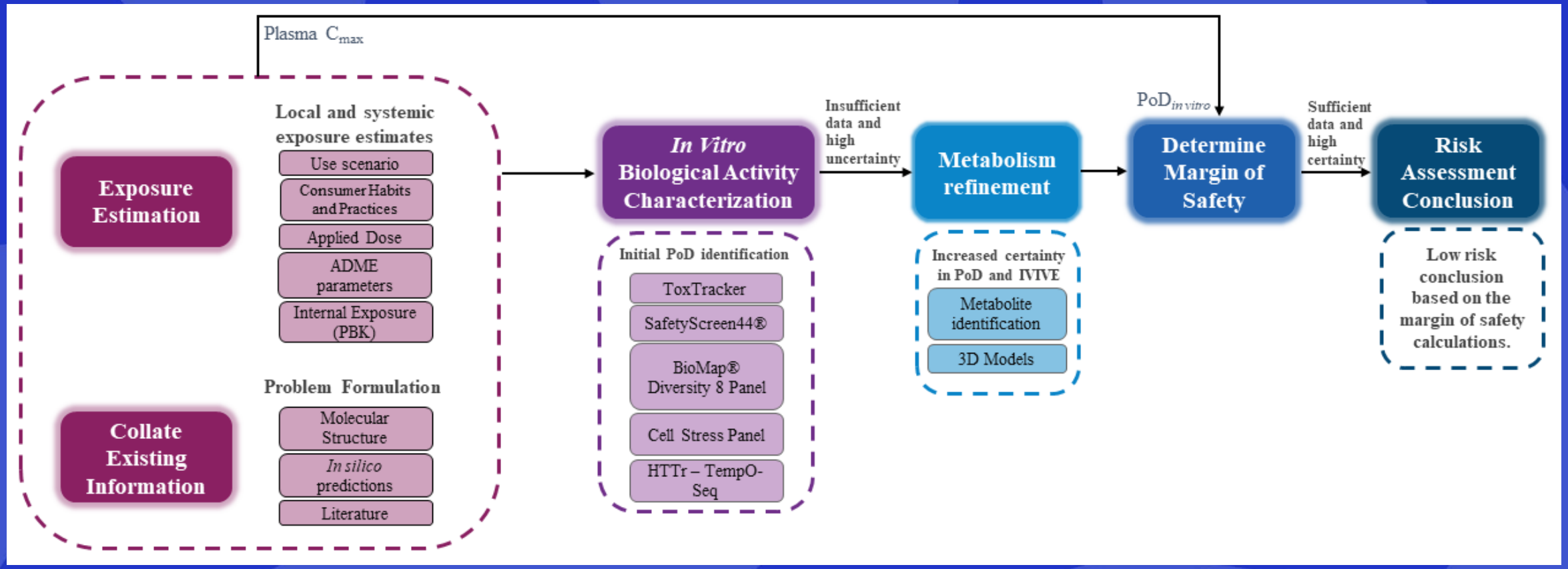
Case Study Approach… Imagine we have no data for: Coumarin

Safety assessment required for 0.1% coumarin in Body Lotion

Safety assessment required for 0.1% coumarin in Face Cream

Baltazar *et al.*, (2020) *Tox Sci* https://doi.org/10.1093/toxsci/kfaa048

Case Study Framework



Baltazar *et al.*, (2020) *Toxicological Sciences* 176(1): 236-252
https://doi.org/10.1093/toxsci/kfaa048

Collection of Existing Data and ADME Parameters

Name	Coumarin
CAS	91-64-5
MW	146.14 g/mol
Log P	1.39
Solubility	0.96 mg/mL in phosphate buffer
ECCS Class	Class 2 (Metabolism)
R_{b2p}	0.7
F_{ub}	0.31
Cl_{int}	929 L/h

Chemistry determinations:

- Partition coefficient logP
- Peptide binding potential

In vitro determined:

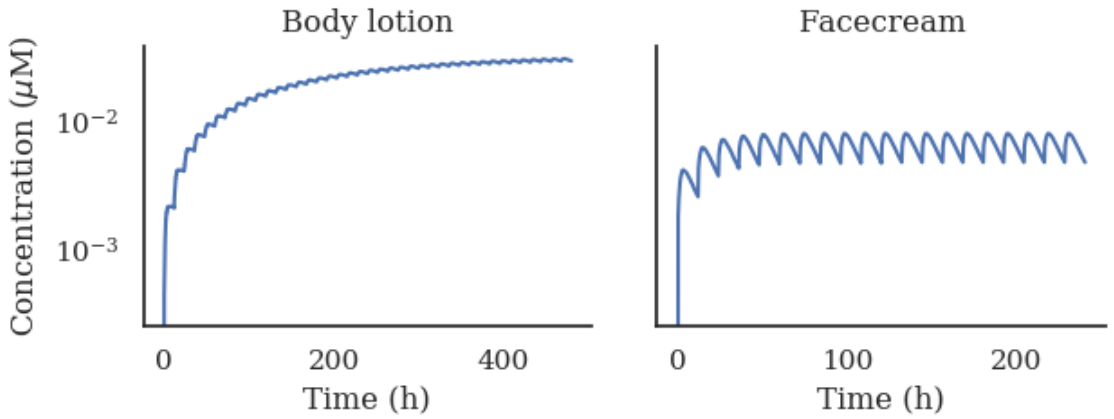
- Kinetic solubility
- Thermodynamic solubility
- Metabolic & chemical stability
- Stability in human plasma
- Plasma protein binding
- Partitioning in blood
- Skin penetration parameters

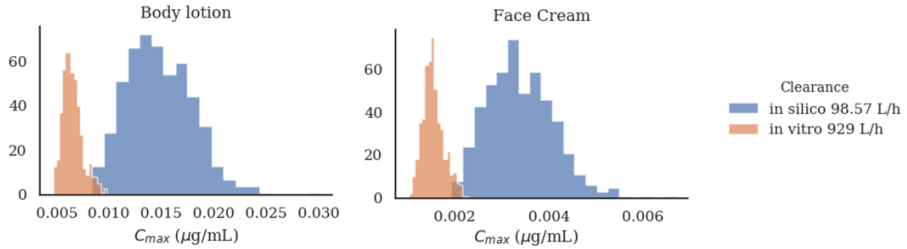
Systemic Bioavailability using PBK Modelling

Key output parameters from uncertainty analysis:

Parameter	Face cream (applied 2x/day)	Body lotion (applied 2x/day)
Plasma Cmax total (µM)	0.023	0.10
95th percentile Cmax (µM)	0.032	0.14

0.1% Face cream & body lotion in Europe

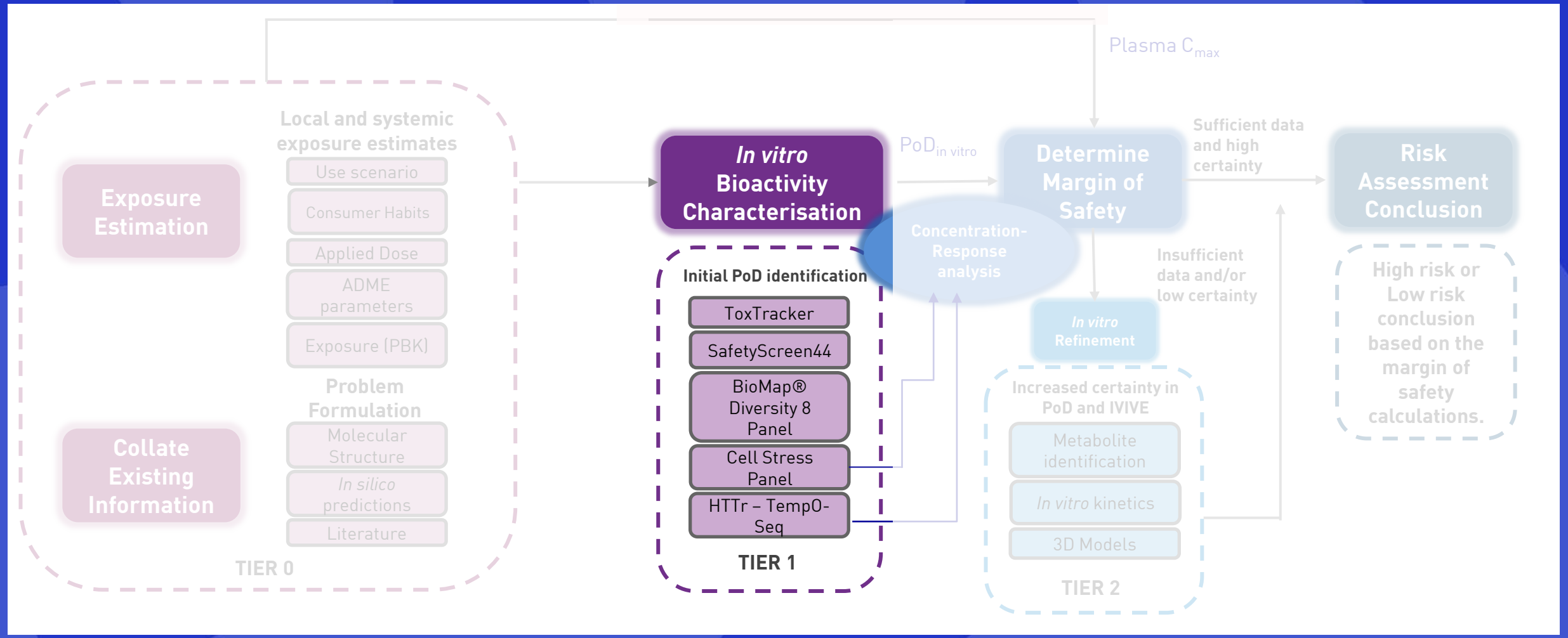




Uncertainty & Population Variability

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.

Ab Initio NGRA Framework

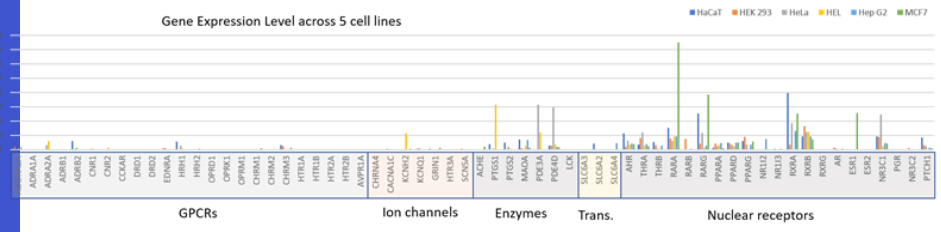


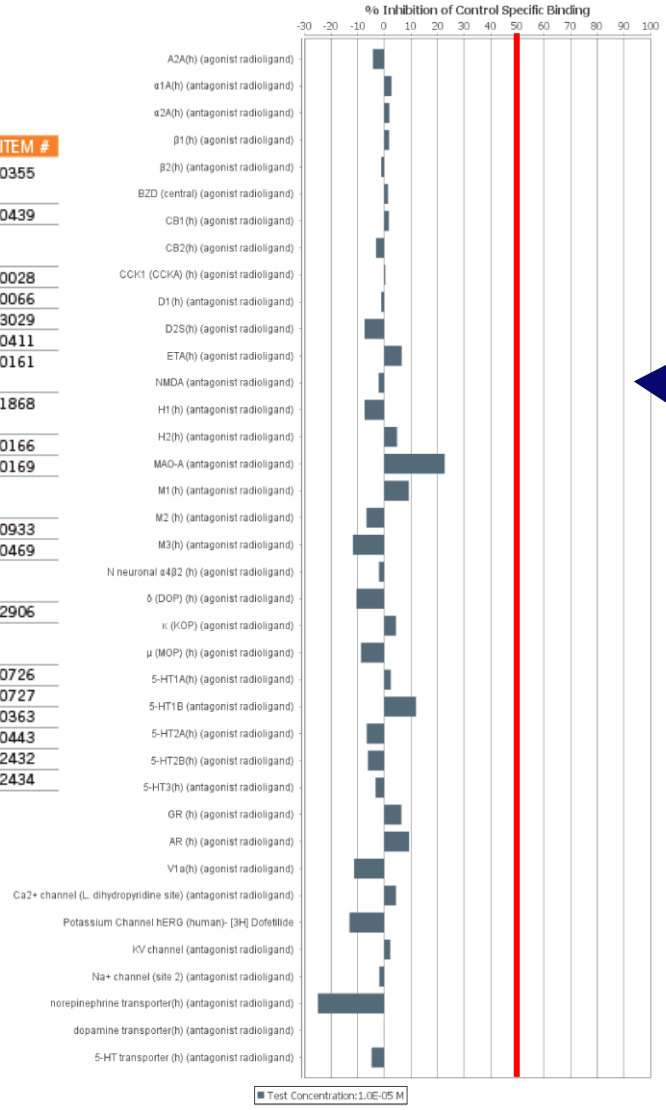
In Vitro Bioactivity: Safety Screen

eurofins | Cerep

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

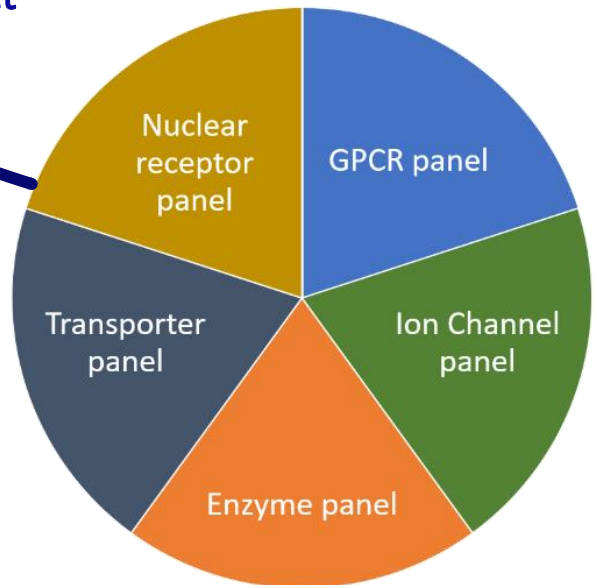
FAMILY	ASSAY	FORMAT	ITEM #
■ GPCR			
ADENOSINE	A_{3A}	•	0004
ADRENERGIC	$alpha_{1A}$		2338
	$alpha_{2A}$		0013
	$beta_1$	•	0018
	$beta_2$	•	0020
CANNABINOID	CB_1	•	0036
	CB_2	•	0037
CHOLECYSTOKININ	CCK_1 (CCK_A)	•	0039
DOPAMINE	D_1		0044
	D_{2L}	•	1322
ENDOTHELIN	ET_A	•	0054
HISTAMINE	H_1		0870
	H_2		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

No receptor/target-led pharmacological effect



SafetyScreen44™ Panel

In Vitro Bioactivity: Cell Stress Panel

Hatherell et al., 2020 *Tox Sci* 176(1): 11-33 https://doi.org/10.1093/toxsci/kfaa054

~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

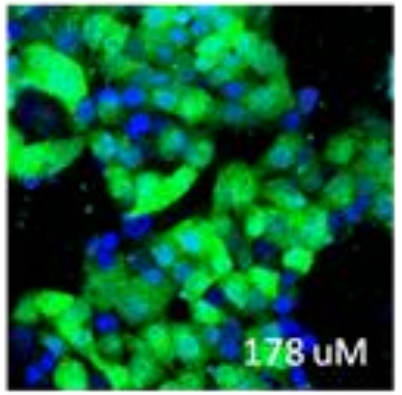
Selection of *in vitro* concentrations based upon realistic human exposures

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

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

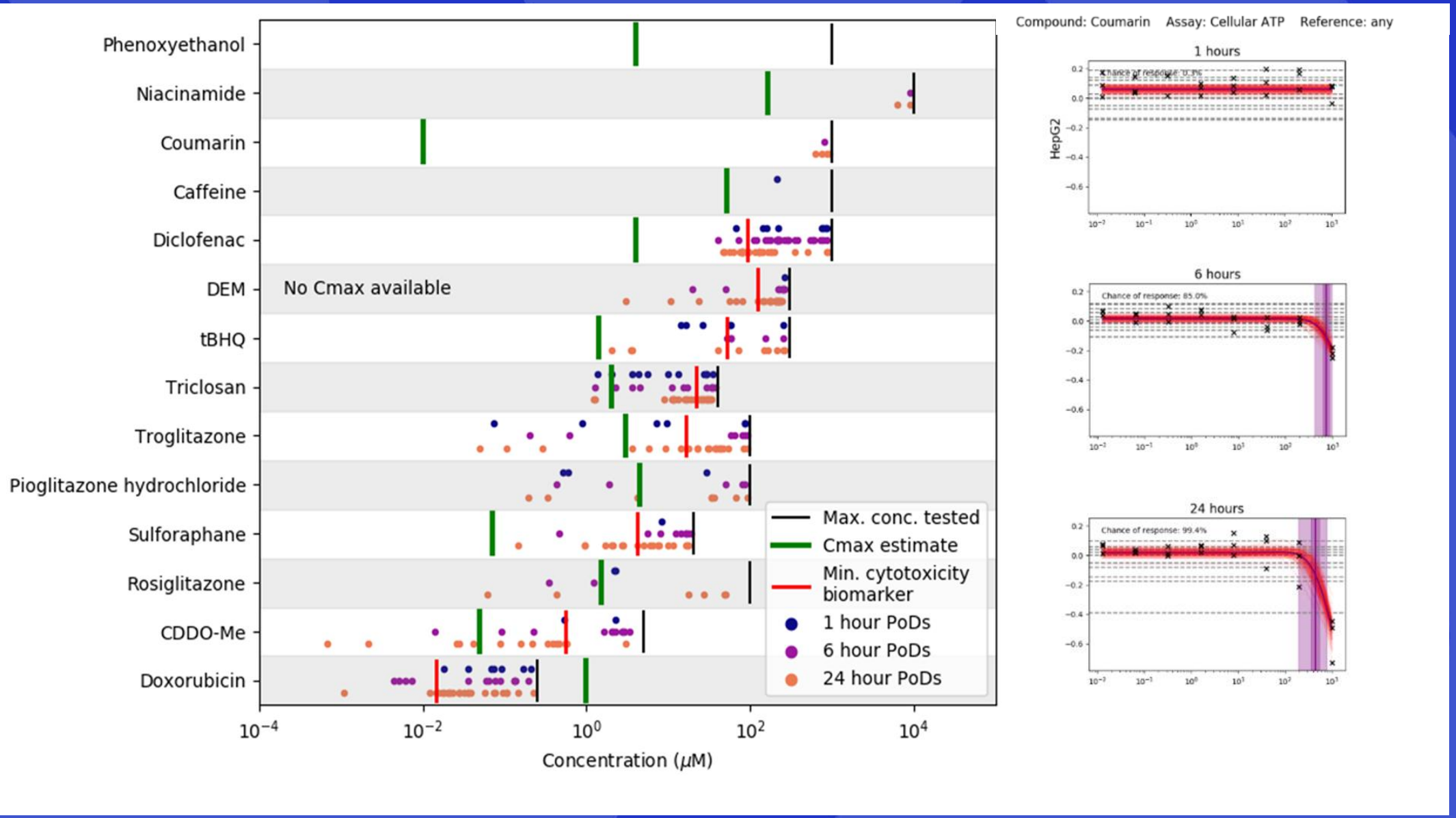
Key
- Exposure scenario adopted for chemical is 'high risk' (from consumer goods perspective).
- Exposure scenario adopted for chemical is 'low risk' (from consumer goods perspective).

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



*now conducted in HepaRG spheroids

In Vitro Bioactivity: Cell Stress Panel

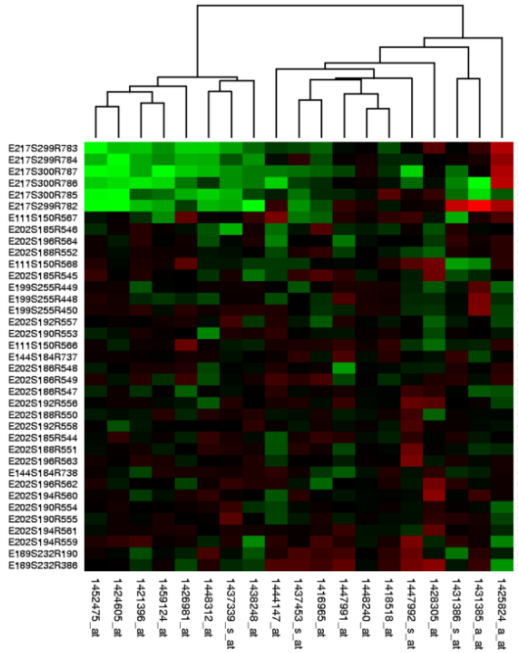


In Vitro Bioactivity: Tempo-Seq Technology

High-Throughput Transcriptomics Gene Expression Profiling (HTTr)

1. Defining a safe operating exposure for systemic toxicity using a **NOTEL** (No Transcriptional Effect Level)
2. Defining compound similarity grouping (Read Across)

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)

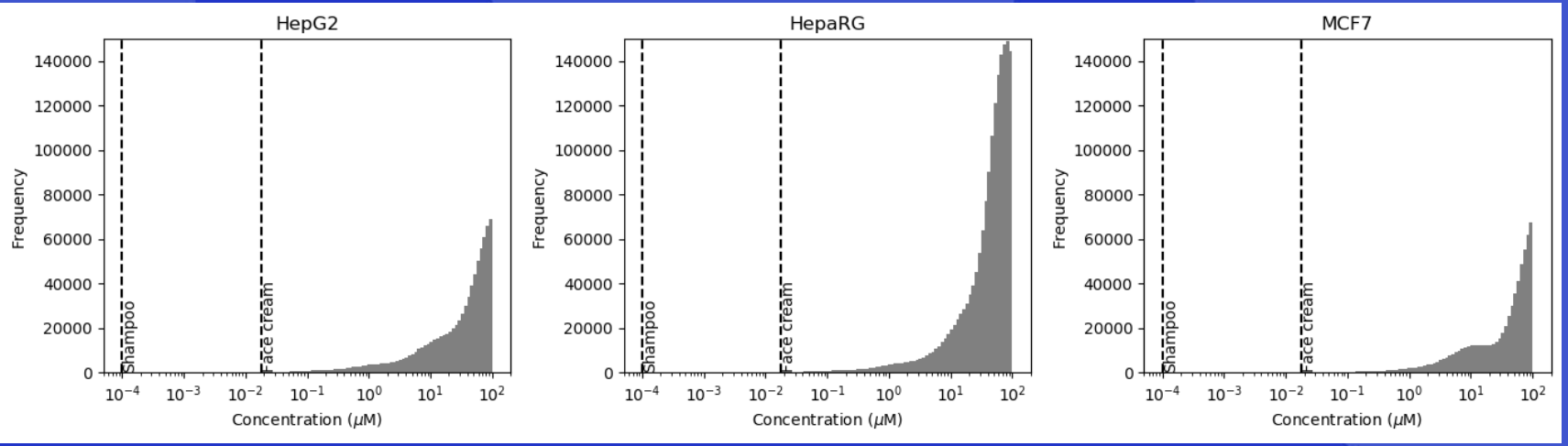
MCF-7 – human breast adenocarcinoma cell line

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

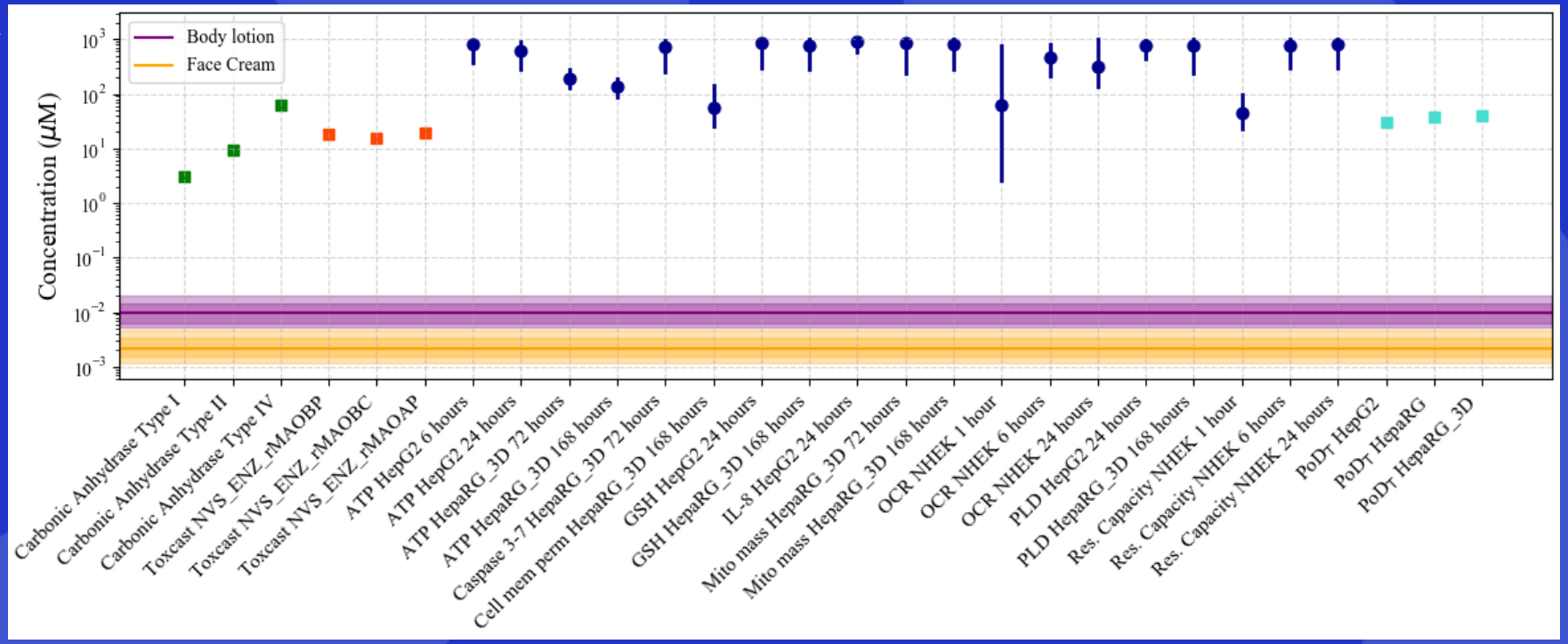
Margin of Safety considering PODs and Exposure

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

C_{max} expressed as a distribution:

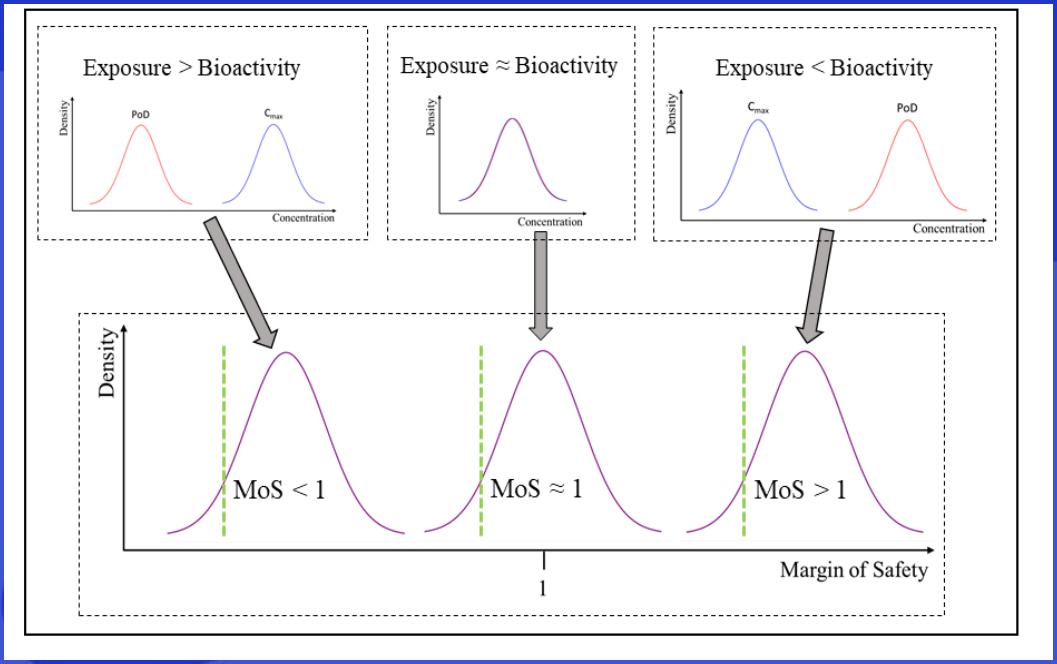
- Line = median (50th percentile)
- Inner band = 25th-75th percentile
- Outer band = 2.5th-97.5th percentile (95th credible interval)

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 (rat brain	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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Challenges to overcome

- Clarity on the level of protection offered by this approach
  - Bioactivity *vs.* Adversity
- Adequacy of cell lines, timepoints, study designs
- Role of metabolism
- Translating principles to other sectors/chemistries
  - Regulation keeping pace with science

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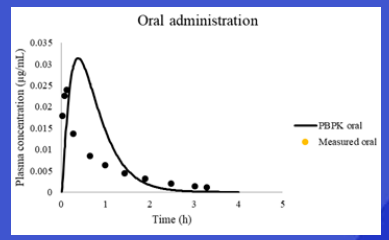
Evaluating the level of protection

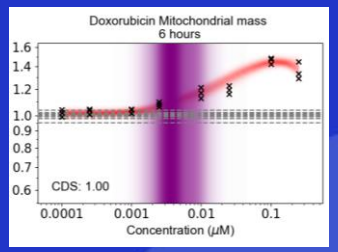
Chemical exposures scenarios

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

Define typical use-case scenarios benchmark chemical-exposures



PBK models of systemic exposure



Calculate the PoDs

Calculate margin of safety

Evaluating the level of protection

Chemical exposures scenarios

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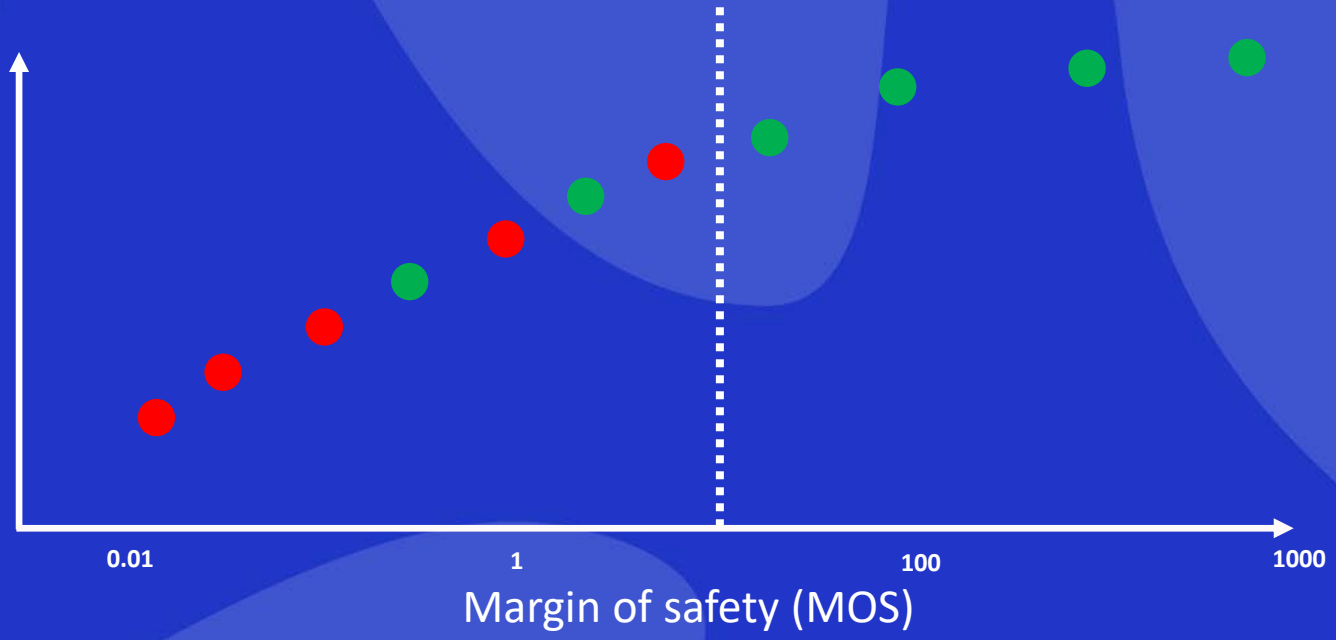

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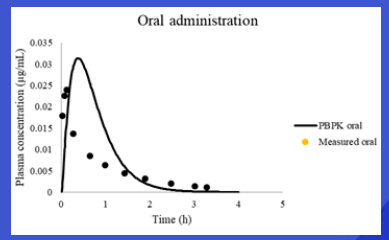

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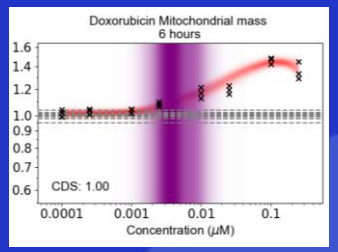

Calculate the PoDs

Calculate margin of safety

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Translation into global requirements

- Once we understand the level of protection and where the approach falls down we can consider translation into requirements
  - Bioactivity/Exposure screen instead of arbitrary tonnage-driven information requirements
  - Beyond cosmetics

Conclusions

- We are seeing increased pace of development and application of next generation risk assessments in the consumer products industry
- NGRA is exposure-led, hypothesis driven, and requires clear articulation of the risk assessment question
- Progress has been possible with a change in mindset (protection not prediction)
- Once we understand the strengths and limitations why shouldn't the same approach be useful in different contexts?

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