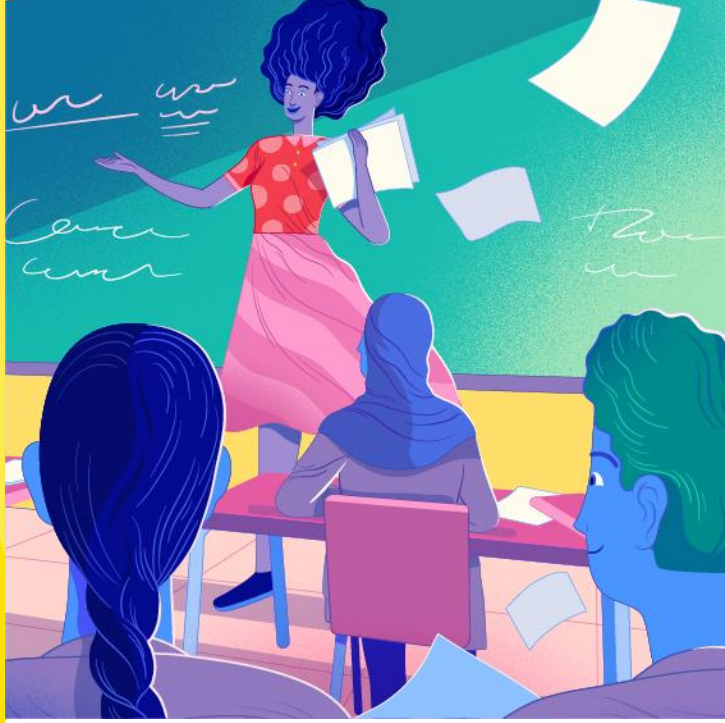
Toxicity testing for the 21st century (TT21C): *Making the transition to Next Generation RISK Assessments (NGRA)*



Maria Baltazar
Safety & Environmental Assurance Centre, Unilever, UK

Unilever

2004-2009 **MSc in Pharmaceutical Sciences**, Faculty of Pharmacy, University of Porto, Portugal

Master's thesis: Feb-Jun 2009 *Developing the model nematode Caenorhabditis elegans as a model organism to study Corynebacterium diphtheria.*
Infection, immunity and microbiology Department, Strathclyde University, Glasgow, UK.

Jun- Oct 2009 **Community Pharmacist**

Oct 2009- Feb 2014 **PhD in Pharmaceutical Sciences- Toxicology Specialty**
"New Formulation of Paraquat with lysine acetylsalicylate: Safety improvement for mammalian and algae species with maintenance of the herbicidal activity"

2011-2013

- **Lecturer** of Biochemical and Molecular Biology Laboratory Methods Applied to Forensic Sciences I (2nd year of BSc degree in Forensic and Criminal Sciences
- **Lecturer** of Food Toxicology Analysis (3rd year of BSc degree in Forensic and Criminal Sciences).

2014-2016 **Toxicologist**-Conduct toxicological risk assessments of ingredients and materials for used in or in contact with tobacco products (including smokeless) and non tobacco products (e-vapour and food supplements)

2016-2020

Various roles!!

- Started as Safety Scientist allergy & immunology
- 2017-2018- Project Leader for Inhalation Toxicity and NGRA case studies
- 2018-2020- Science Leader for Systemic toxicity; co project leader for inhalation toxicity; and NGRA case studies communication

Unilever

WE MAKE MANY OF THE WORLD'S FAVOURITE BRANDS

Many products means many ingredients=

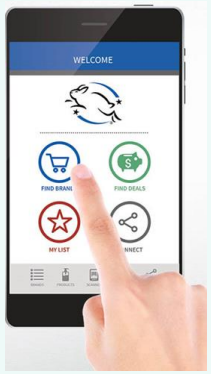
potential for impact on the health of consumers & environment=

Need for robust safety assessment of ingredients in consumer products

- More than 300 new patent applications filed each year
- A portfolio of more than 20,000 patents and patent applications
- Total > 400 Brands

Unilever

Increasing numbers of global consumers want their consumer products not tested on animals+ transparency



Scientific, societal, regulatory and ethical reasons are demanding change; calls for non-animal, next generation risk assessments

PETA Cruelty-Free and Vegan

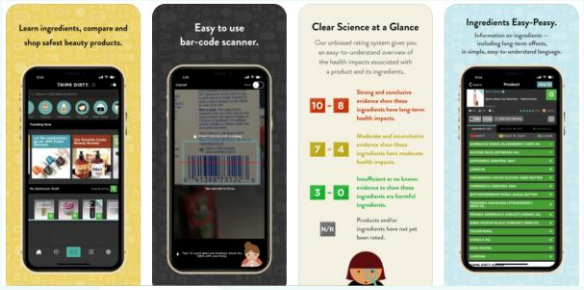
PETA Cruelty-Free

EUROPEAN VEGETARIAN UNION V-LABEL.EU VEGAN

NOT TESTED ON ANIMALS

NO CRUEL COSMETICS

Shop Cruelty-Free www.LeapingBunny.org



Unilever

RISK ASSESSMENT GOAL: Can we use a new ingredient safely?

Can we safely use X% of ingredient Y in product Z?

All safety assessments of cosmetic ingredients are exposure-driven:

Consumer Exposure

Potential hazards of the ingredients

Risk Assessment

Unilever

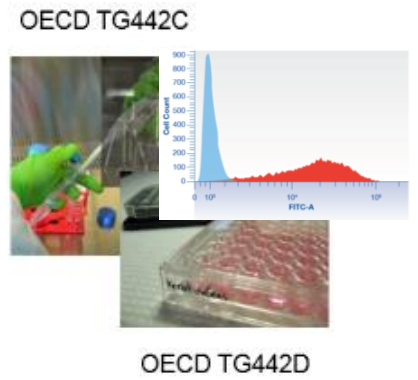
Maximising use of existing information and non-animal approaches

1. All available safety data
2. *In silico* predictions
3. Exposure-based waiving approaches
4. History of safe use*
5. Read across
6. Use of *existing OECD in vitro approaches*

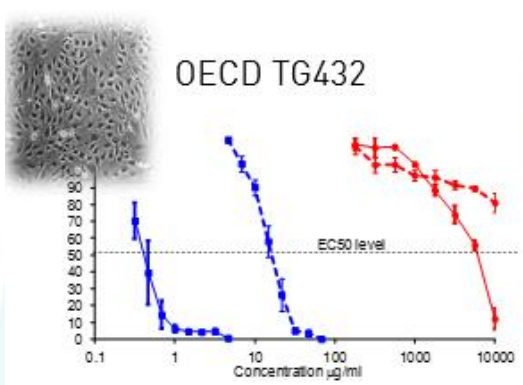
(Skin and eye irritation; skin sensitization; phototoxicity; mutagenicity)

OECD test methods

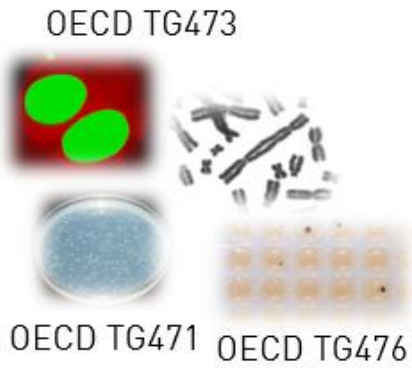
Skin and eye irritation



Skin sensitisation



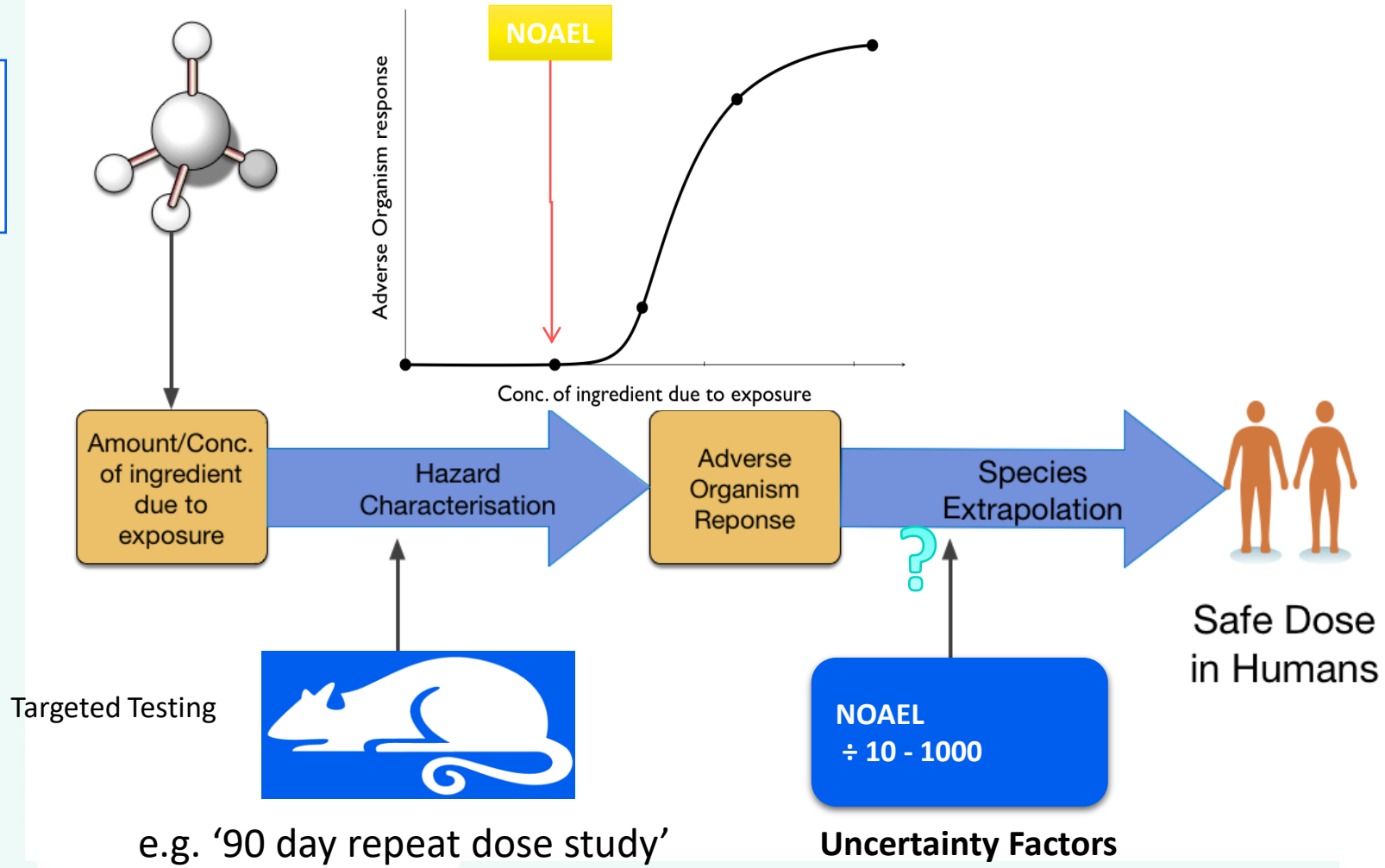
Phototoxicity



Genotoxicity

What about systemic toxicity?

Is the molecule safe?



e.g. '90 day repeat dose study'

Uncertainty Factors

2007-2018: using 21st century science

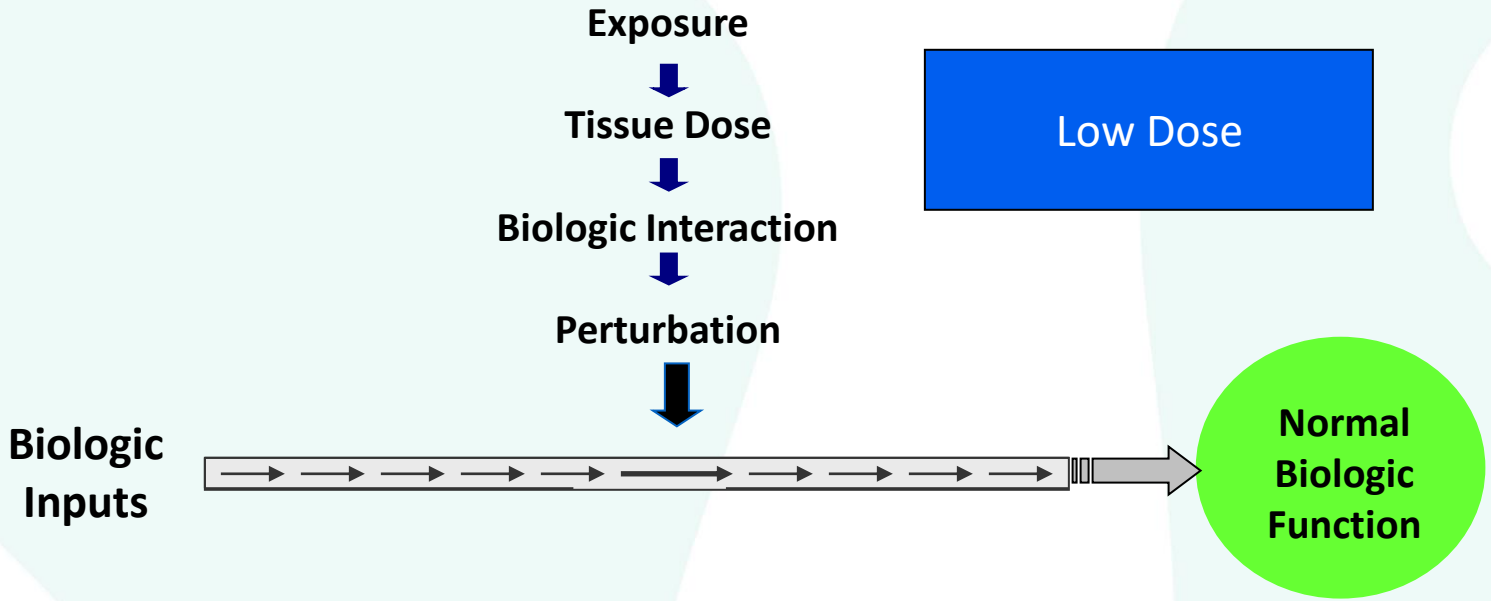
2007 – Toxicity testing in the 21st century (TT21C)

Perturbation of 'toxicity pathways' and stress responses

"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.**" 2007

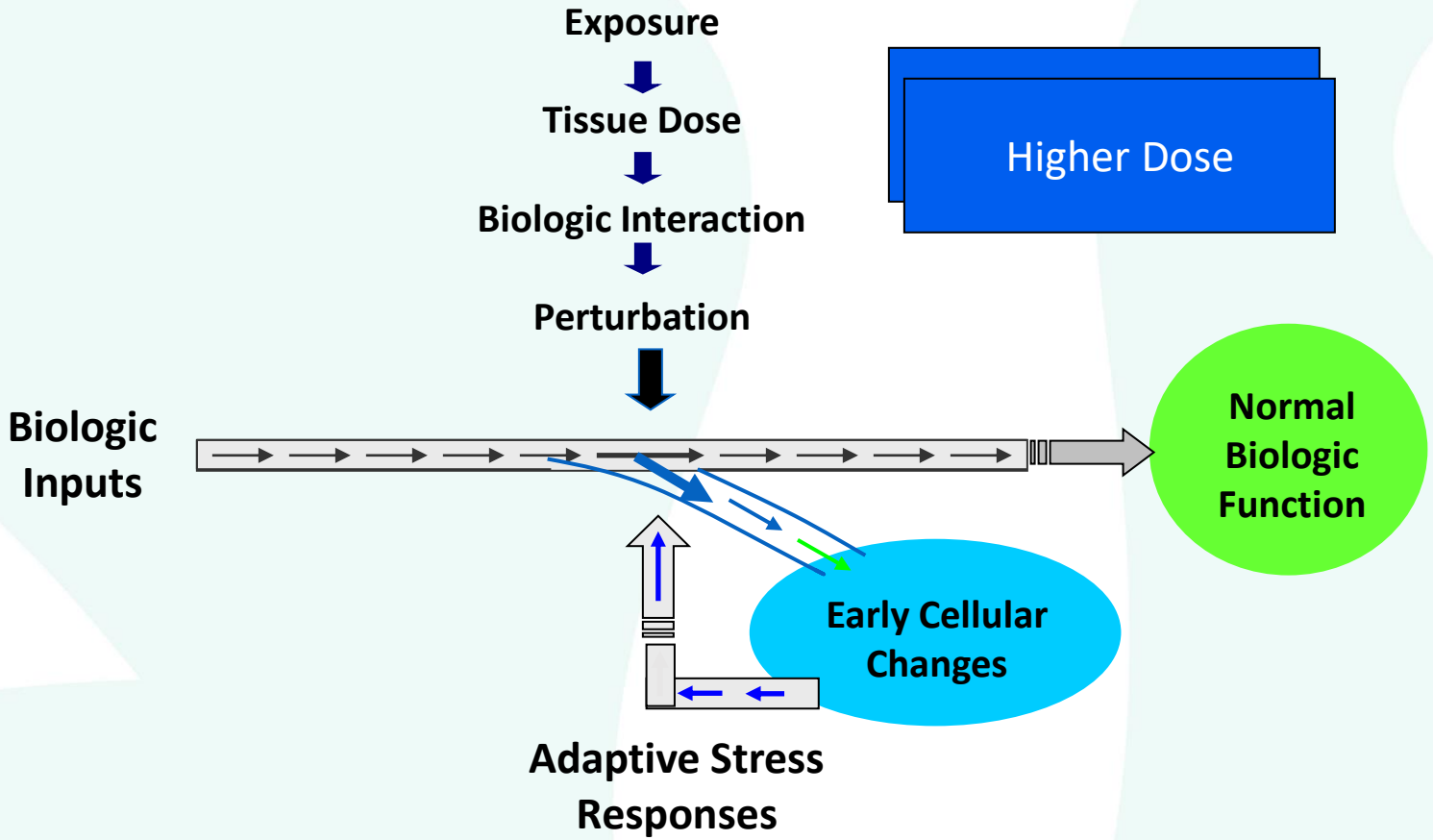
Tox21/ToxCast
~700 HTS Biological Pathways Assays

Based on perturbation of 'toxicity pathways'



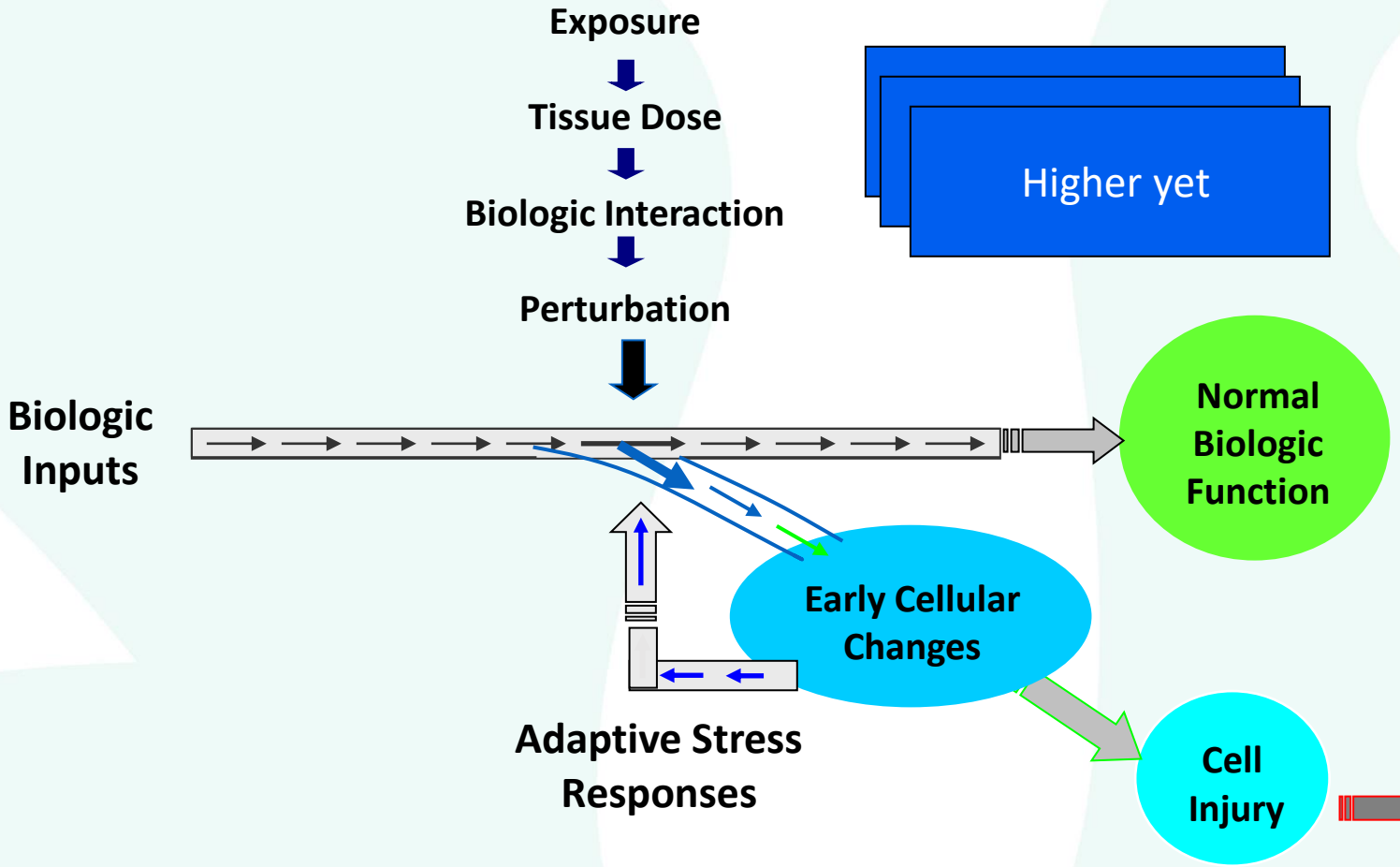
Krewski. J Toxicol Environ Health B Crit Rev. 2010 Feb;13(2-4):51-138

Based on perturbation of 'toxicity pathways'



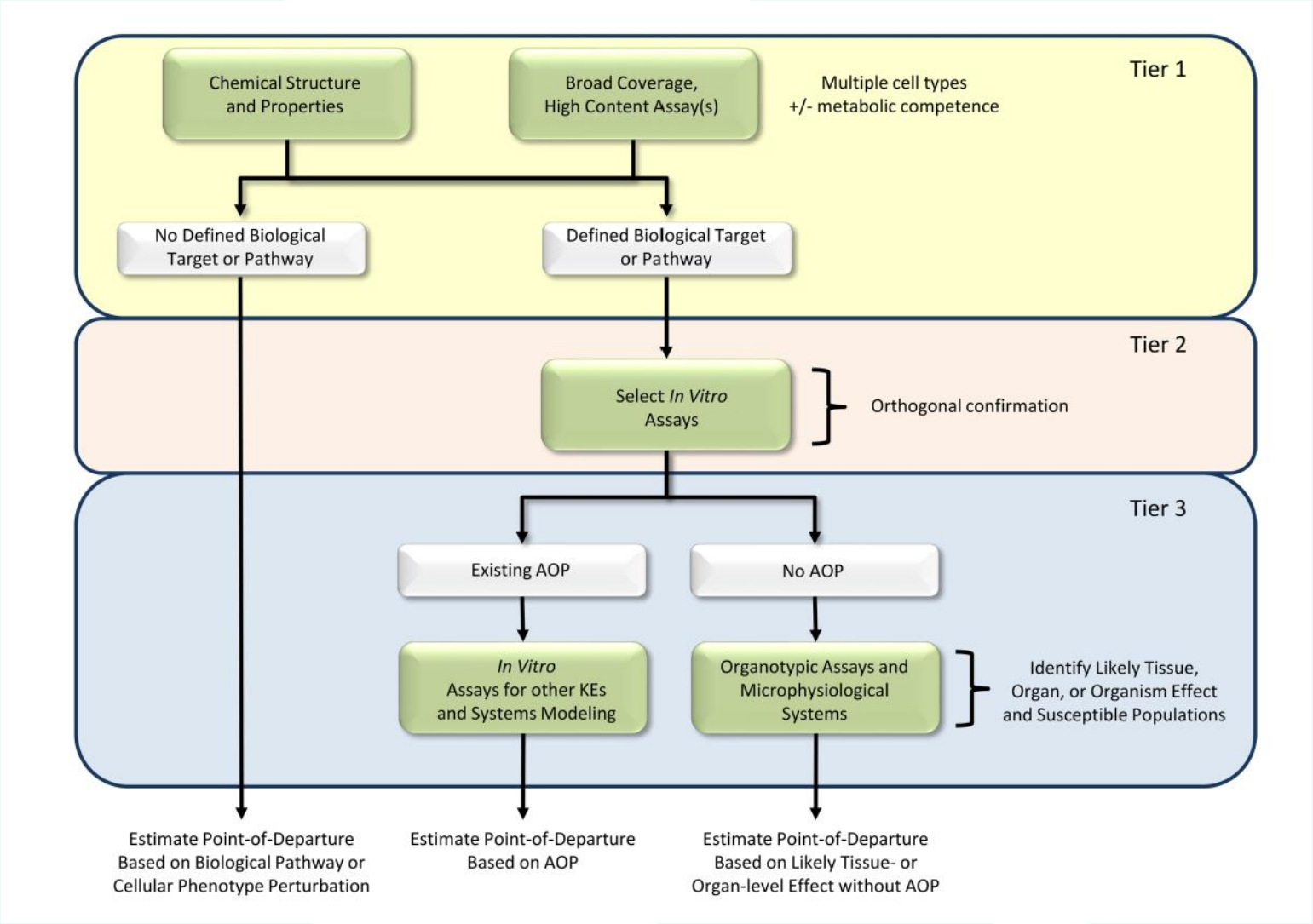
Krewski. J Toxicol Environ Health B Crit Rev. 2010 Feb;13(2-4):51-138

Based on perturbation of 'toxicity pathways'



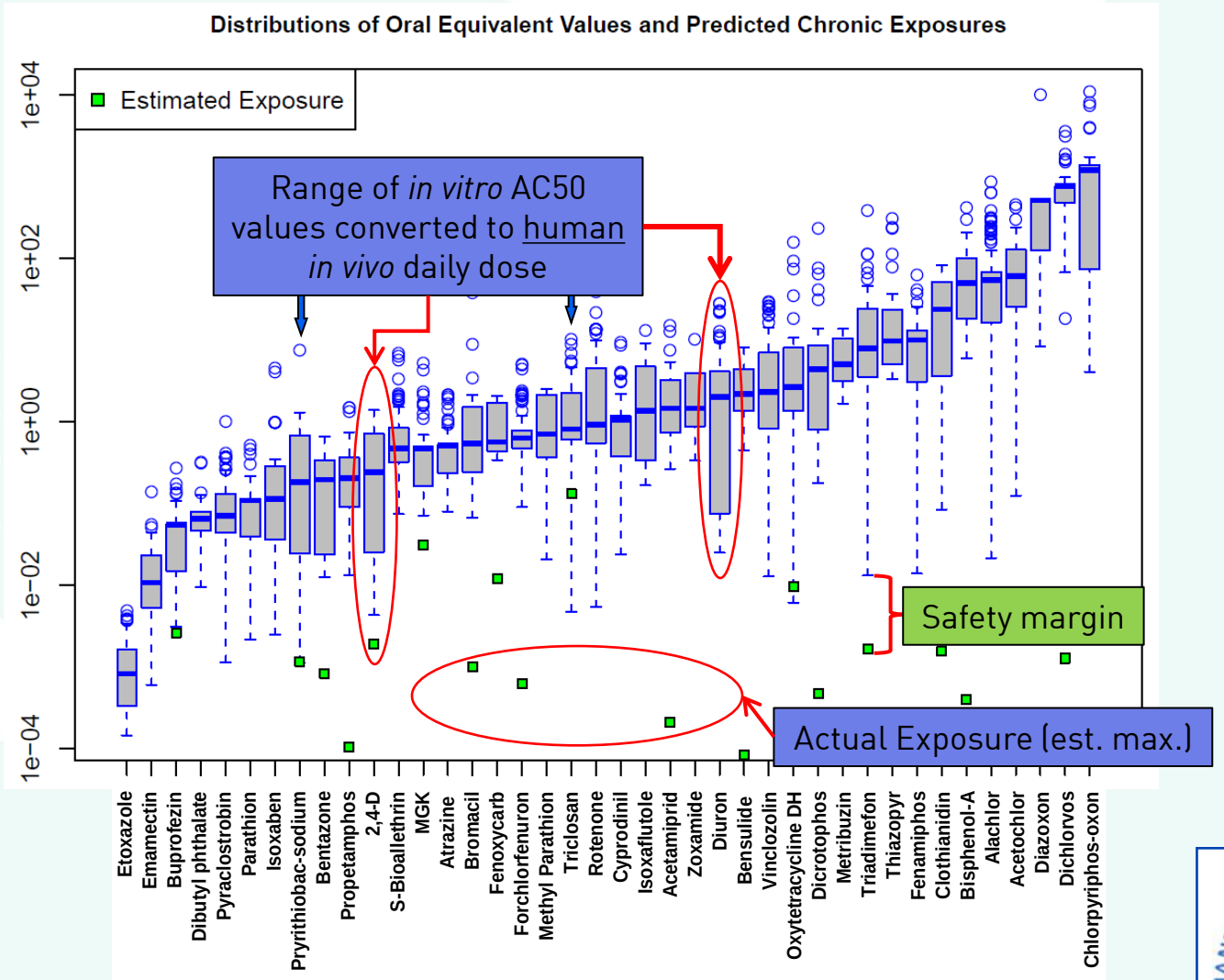
Krewski. J Toxicol Environ Health B Crit Rev. 2010 Feb;13(2-4):51-138

Example of a tiered testing framework for hazard characterization- US EPA



Russell S Thomas et al., 2019. The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency. Tox Sci 169(2):317-332.

In Vitro Bioactivity vs Bioavailability- Protection not Prediction



Slide from Dr Rusty Thomas, EPA, with thanks

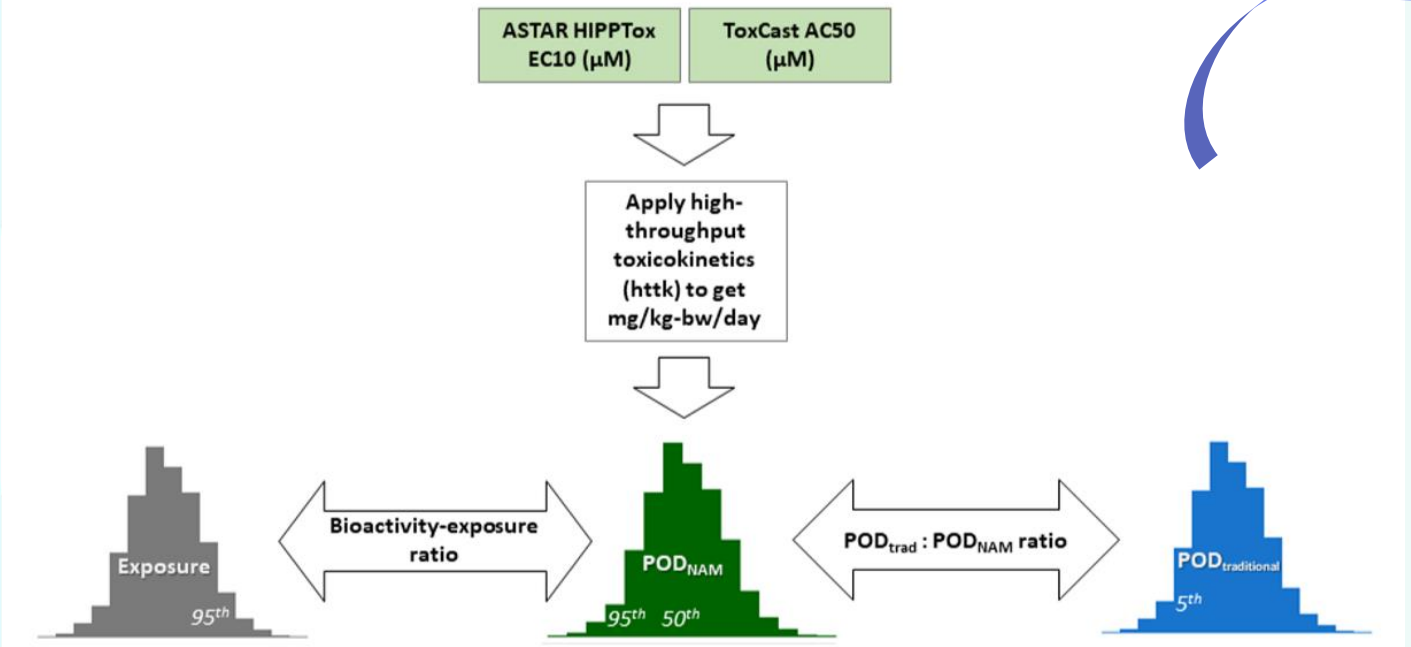
Rotroff, *et al.* Tox.Sci 2010

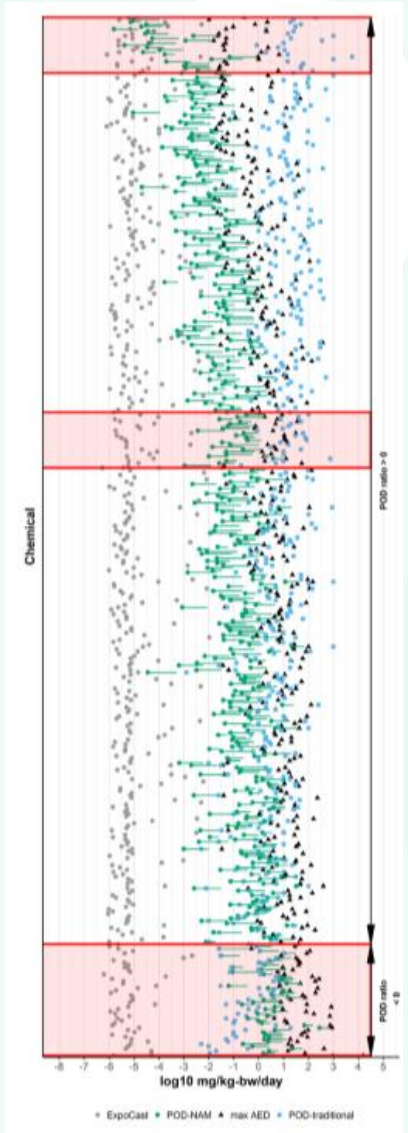
The philosophy behind this type of risk assessment aimed at preventing harm is **based on the premise of "Protection not Prediction".**

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.**

Recent research has shown that for 417 out of 448 chemicals tested the point of departure derived (PoD) from NAMS was more conservative than the in vivo PoD

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



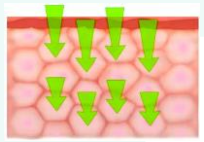


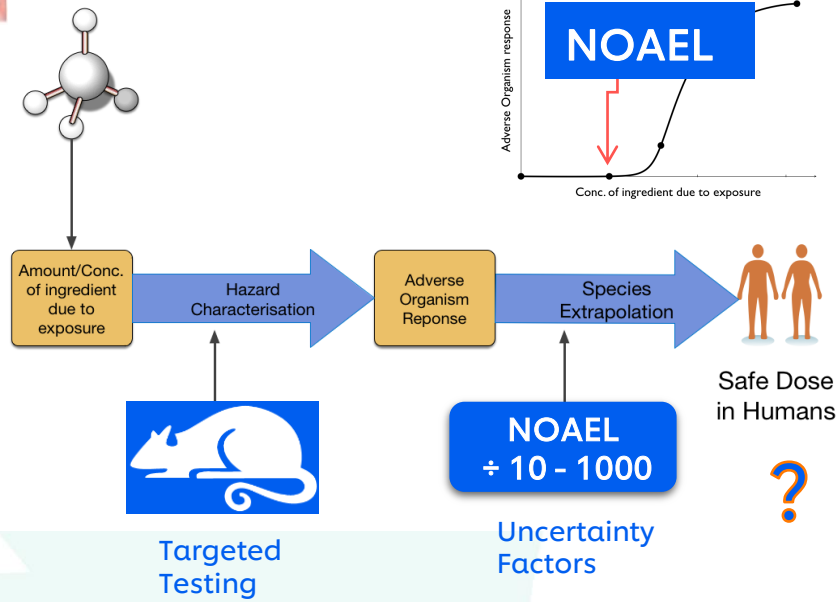
414/448 chemicals = *92% of the time this naïve approach appears conservative*

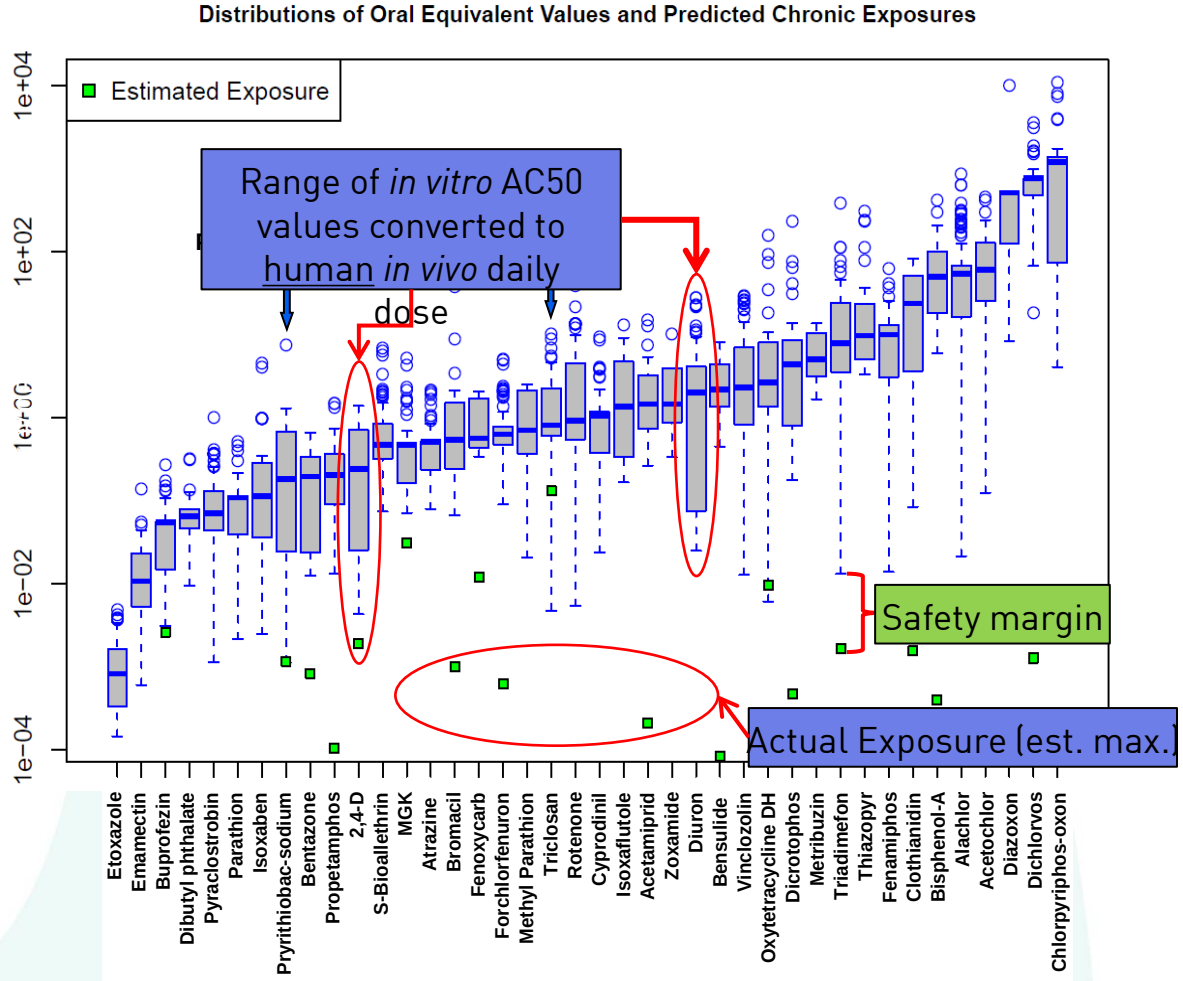
Katie Paul-Friedman *et al.* 2019 *Tox Sci* 173(1): 202-225

The margin of safety (MoS) approach and decision making

Is it safe?

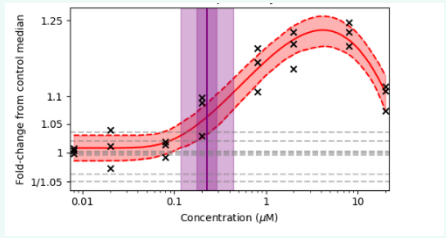






NGRA: the margin of safety (MoS) approach and decision making

Point of departure derived from concentration-response data

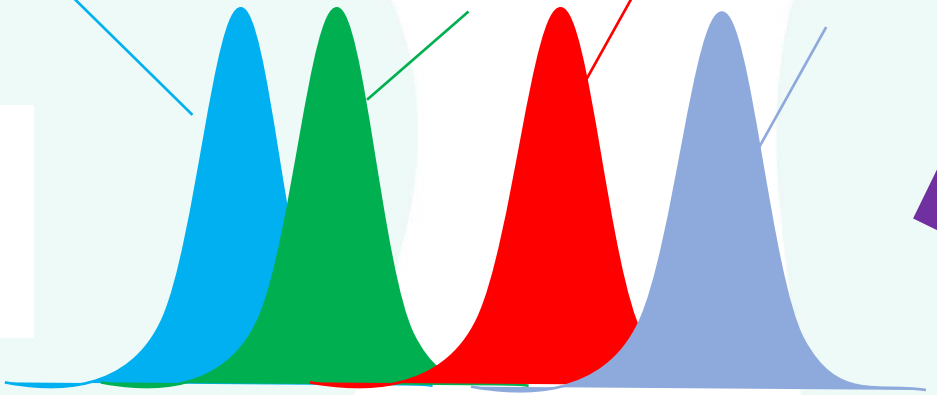


Cellular stress assays

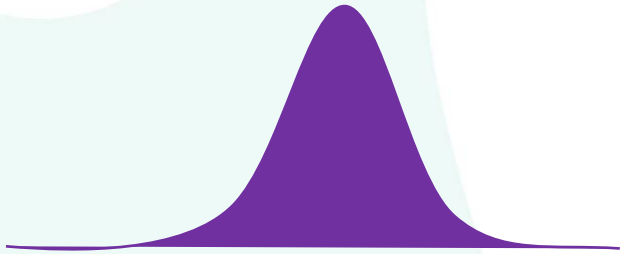
Transcriptomics

Receptor binding

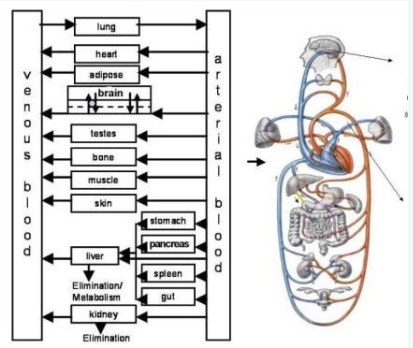
Others



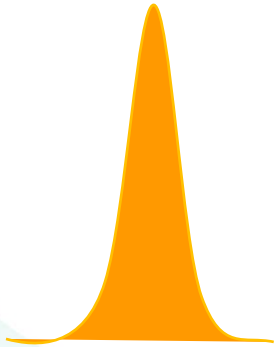
Calculation of Margin of Safety (MoS) distribution



Exposure models (PBK, free/total concentration)

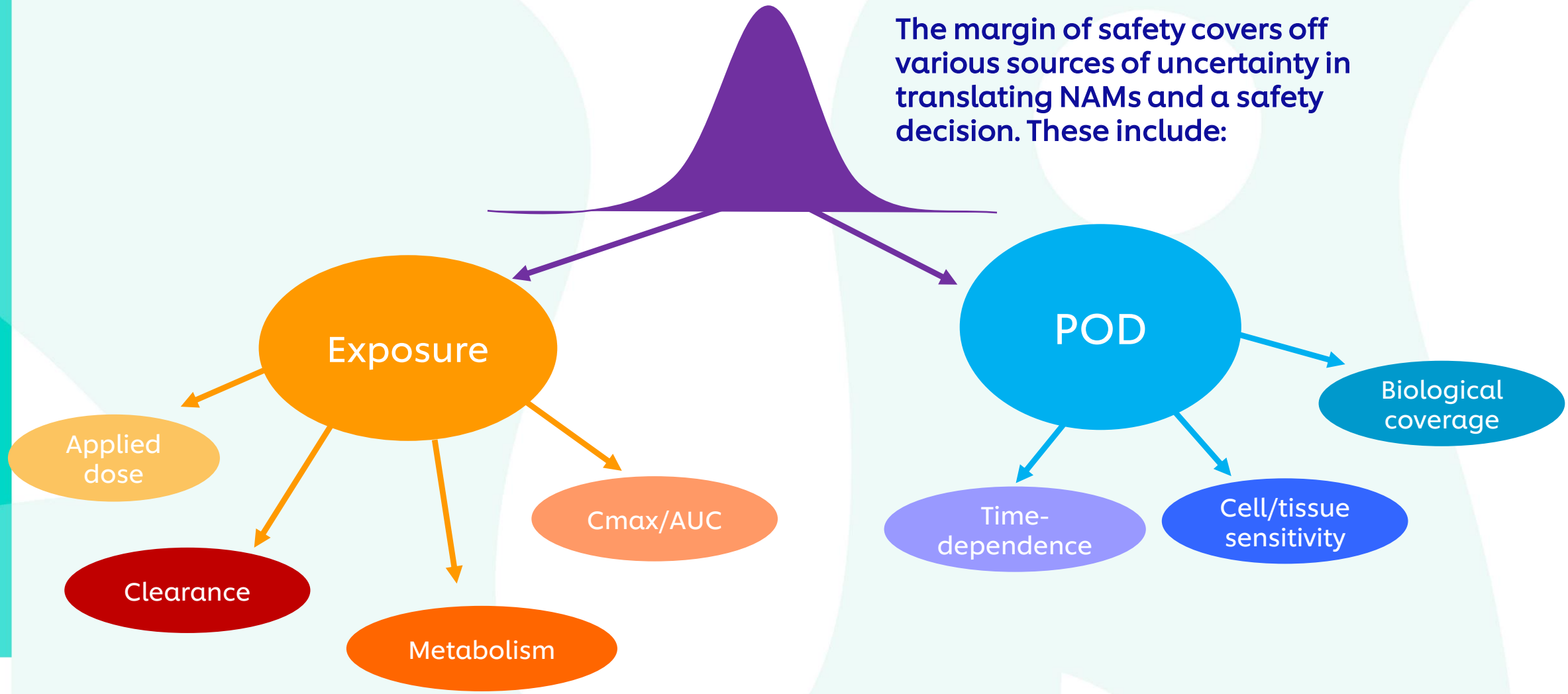


Exposure estimation: Plasma C_{max}



The MoS is defined as the ratio the PoD and the relevant plasma C_{max} estimate

NGRA: Sources of uncertainty should be characterized and documented



Introduction to Next generation risk assessment (NGRA)

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

ICCR
9 principles of NGRA

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

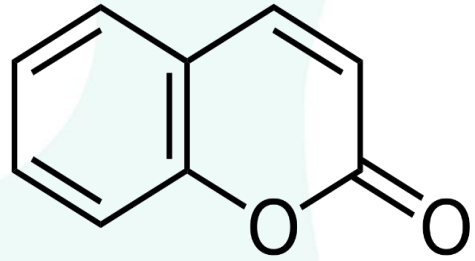
Principles describe how a NGRA should be conducted:
Following an appropriate appraisal of existing information
Using a tiered and iterative approach
Using robust and relevant methods and strategies

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. Computational Toxicology Volume 7, August 2018, Pages 20-26

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

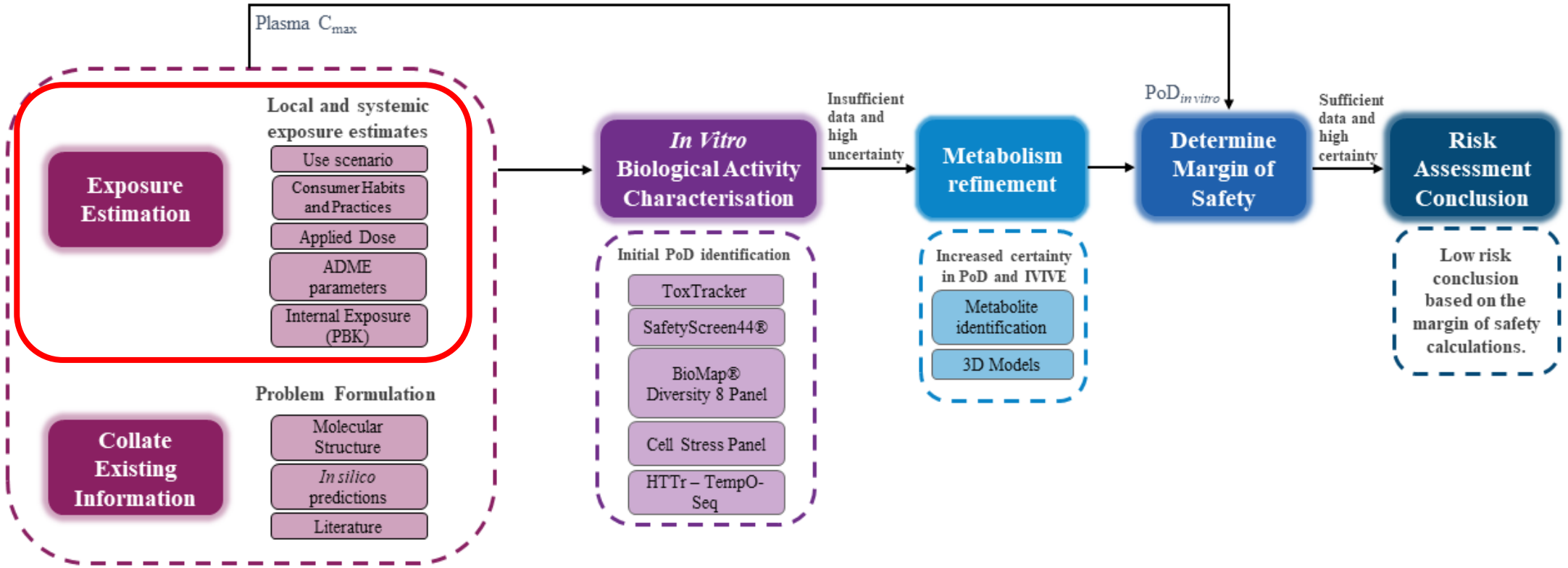
0.1% COUMARIN IN FACE CREAM FOR EU MARKET (NEW FRAGRANCE)



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

Next-Generation Risk Assessment case study workflow for 0.1% coumarin in face cream

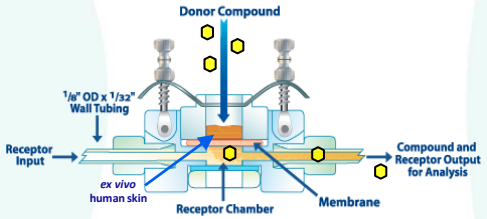


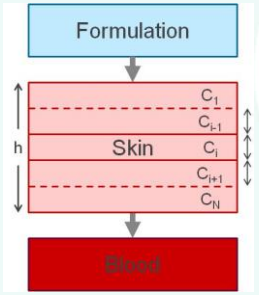
Baltazar *et al.*, (2020) *Tox Sci* (in press)
https://doi.org/10.1093/toxsci/kfaa048

NGRA: The assessment is exposure-led

- Route of exposure
- Consumer use (Habits &Practices)
- Applied dose (external concentration)

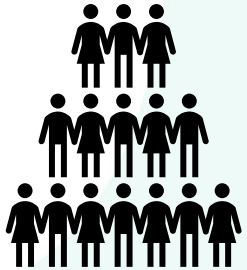
ADME parameters



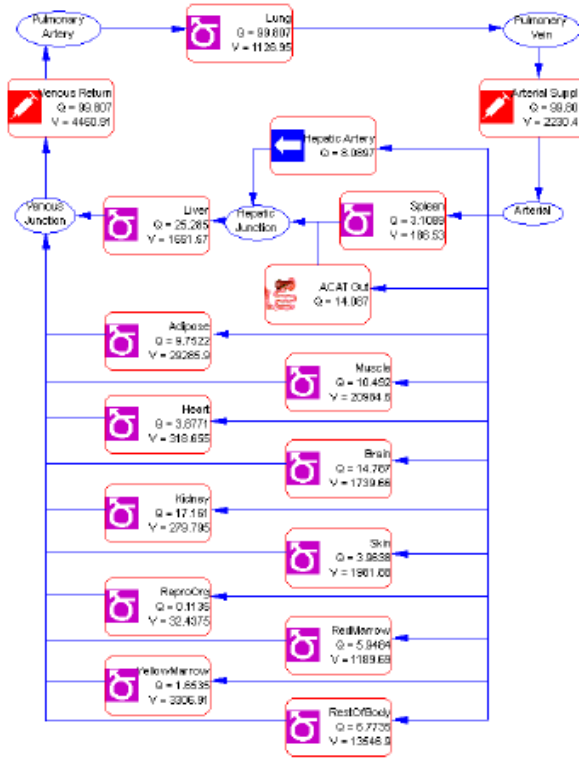


- Skin penetration
- Phys-chem properties
- Hepatic clearance
- Fraction unbound
- blood:plasma ratio

Uncertainty analysis- Population simulation



Physiologically-based kinetic (PBK) modelling – Internal concentration (plasma, urine, organ-level)



NGRA for 0.1% coumarin in face cream: exposure estimation

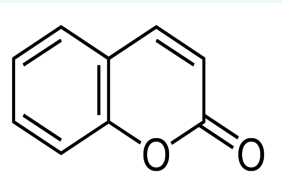
Creme GLOBAL

Table 2: Estimated daily exposure levels for different cosmetic product types according to Cosmetics Europe data (SCCNFP/0321/00; Hall *et al.*, 2007, 2011).

Product type	Estimated daily amount applied	Relative amount applied (mg/kg bw/d)	Retention factor [1]	Calculated daily exposure (g/d)	Calculated relative daily exposure (mg/kg bw/d)
Bathing, showering					
Shower gel	18.67 g	279.20	0.01	0.19	2.79
Hand wash soap [2]	20.00 g	-	0.01	0.20 [3]	3.33
Hair care					
Shampoo	10.46 g		0.01	0.11	1.51
Hair conditi...			...01	0.04	0.60

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

Assessment is exposure-led and uses available habits and practices data

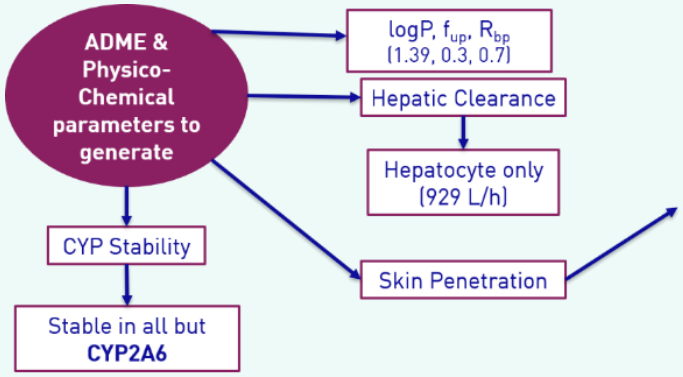


Parameter	Face cream
Amount of product used per day (g/day) using 90th percentile	1.54
Frequency of use	2 times/day
Amount of product in contact with skin per occasion (mg)	770
Ingredient inclusion level	0.1%
Skin surface area (cm2)	565
Exposure duration per occasion	12 hours
Amount of ingredient in contact with skin per occasion (mg)	0.77
Local dermal exposure per occasion (µg/cm2)	1.36
Systemic exposure per day (mg/kg)	**0.02**

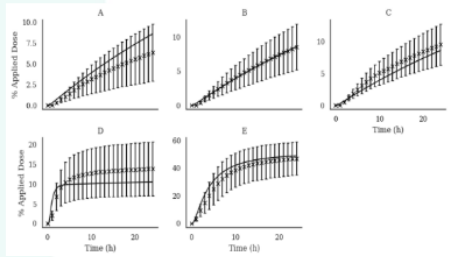
Unilever

NGRA framework: exposure estimation – PBK modelling

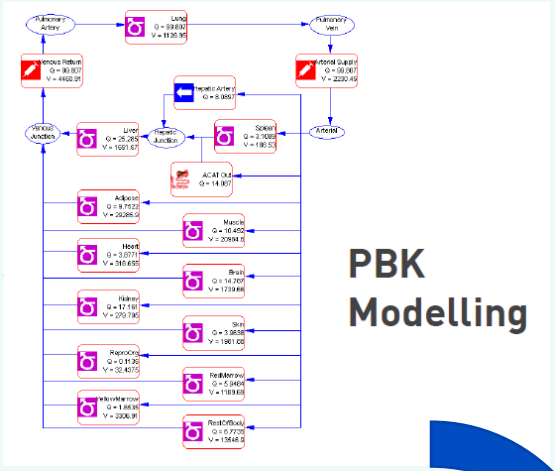
1. *in silico* predictions and *in vitro data* generation on critical parameters



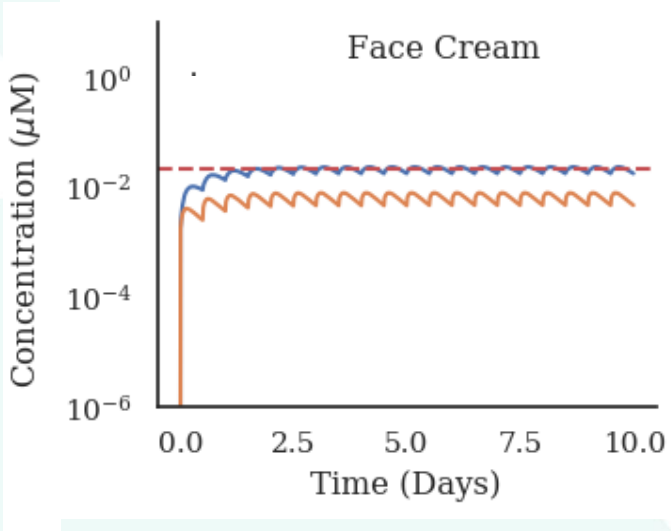
Skin absorption study

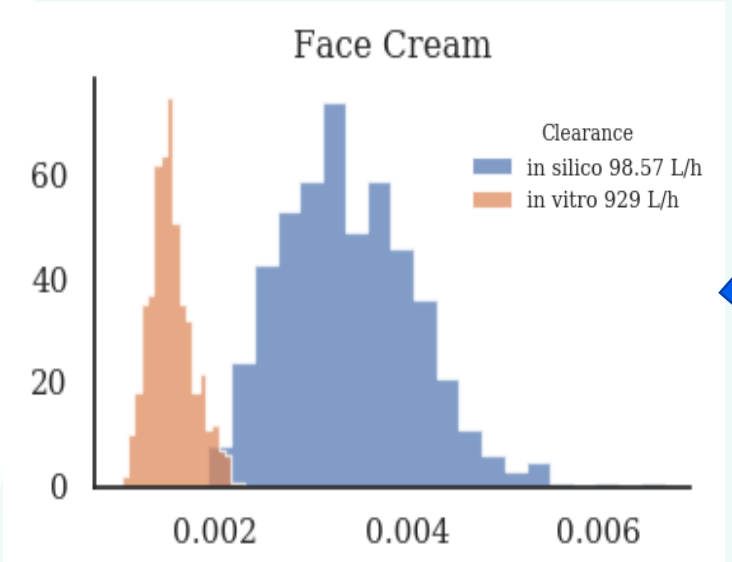


GastroPlus® (Simulations Plus)



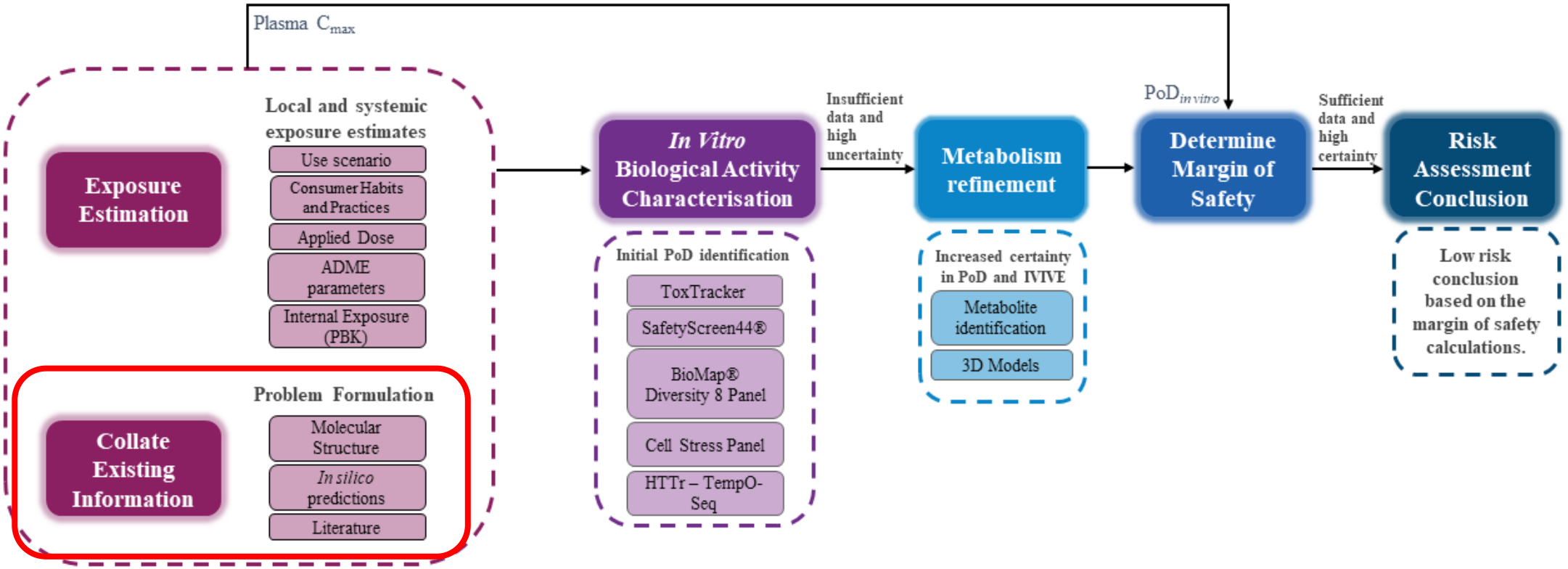
PBK Modelling





Moxon *et al.*, (2020). Application of physiologically based kinetic (PBK) modelling in the next generation risk assessment of dermally applied consumer products. Toxicology in Vitro Volume 63

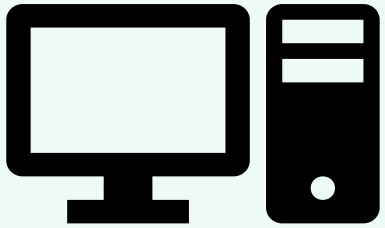
Next-Generation Risk Assessment case study workflow for 0.1% coumarin in face cream



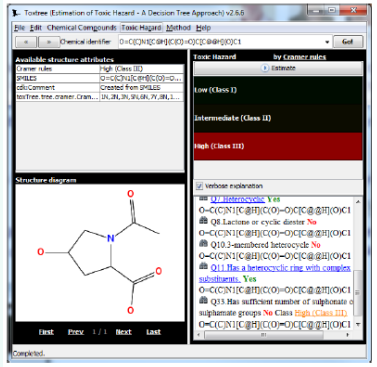
Baltazar *et al.*, (2020) *Tox Sci* (in press)
https://doi.org/10.1093/toxsci/kfaa048

NGRA for 0.1% coumarin in face cream: in silico predictions

In silico tools

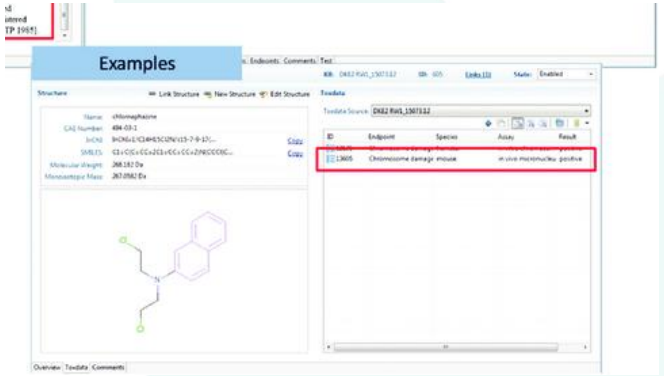


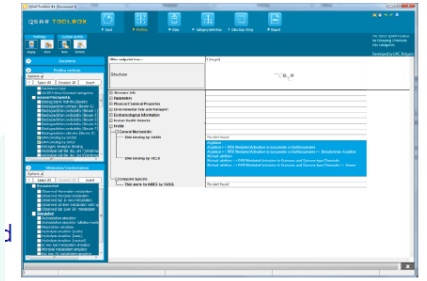
ToxTree



Derek nexus

OECD QSAR TOOLBOX





In silico models to predict Molecular initiating events (MIEs)

OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org ToxSci 20 Years

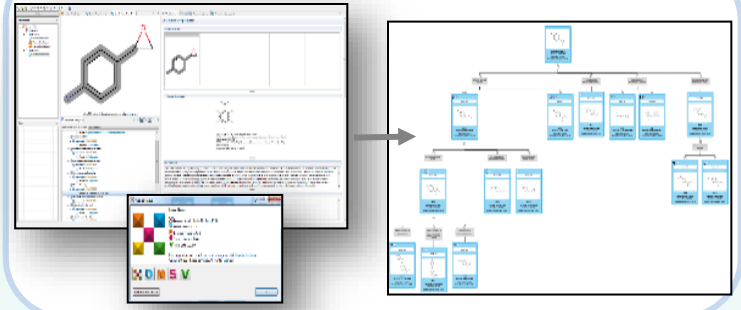
TOXICOLOGICAL SCIENCES, 165(1), 2018, 213–223

doi: 10.1093/toxsci/kfy144
Advance Access Publication Date: July 18, 2018
Research Article

Using 2D Structural Alerts to Define Chemical Categories for Molecular Initiating Events

Timothy E. H. Allen,* Jonathan M. Goodman,*,1 Steve Gutsell,† and Paul J. Russell†

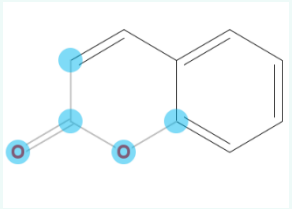
Meteor nexus



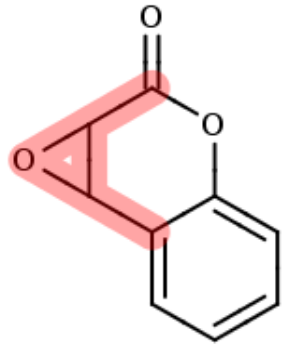
Metabolic fate predictions

Unilever

NGRA for 0.1% coumarin in face cream: in silico predictions



Generation of hypothesis for potential Molecular Initiating events –ToxTree, MIE ATLAS*, OECD toolbox



Initial Hypothesis

- Coumarin might bind to proteins- MIE for induction of skin sensitisation
- DNA binding alert + epoxide formation MIE for genotoxicity
- Reactive metabolites might be formed with alerts for both genotoxicity and skin sensitisation
- No binding alerts for the 39 targets in MIE atlas

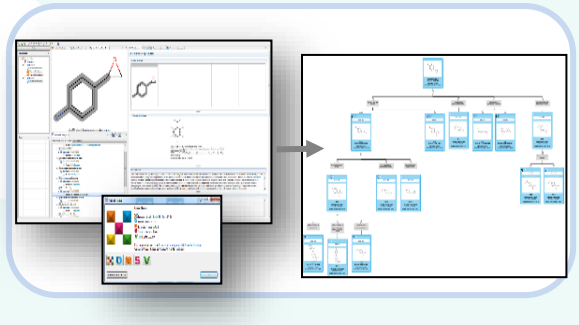
*Allen THE et al., 2018. Using 2D Structural Alerts to Define Chemical Categories for Molecular Initiating Events. Toxicol Sci. 2018 Sep 1;165(1):213-223

NGRA for 0.1% coumarin in face cream: in silico predictions - Metabolism

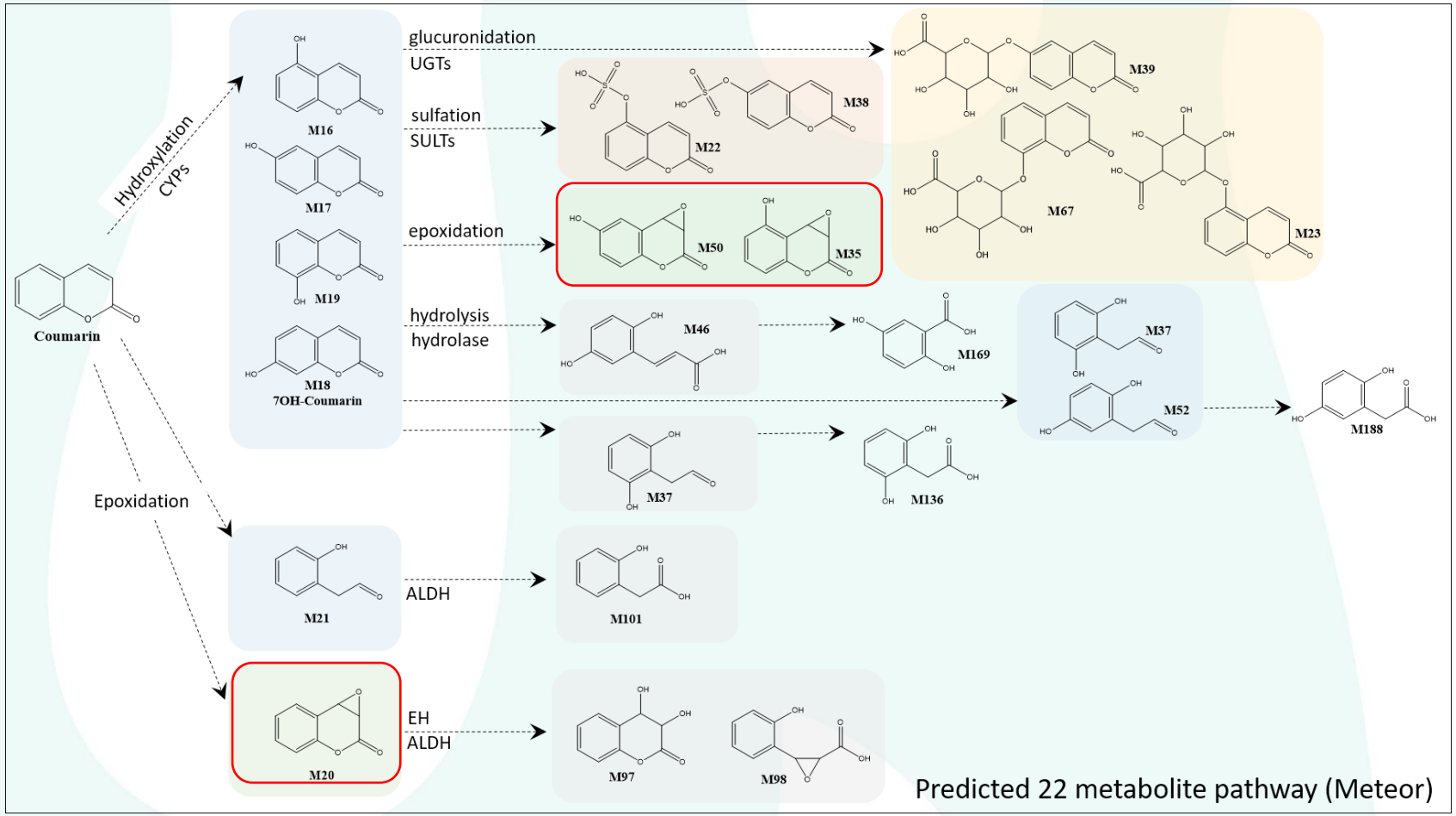
In silico biotransformation

Meteor nexus

Lhasa Limited



- Hydroxylation predicted as main route of biotransformation
- Reactive metabolites (e.g. epoxides) predicted.



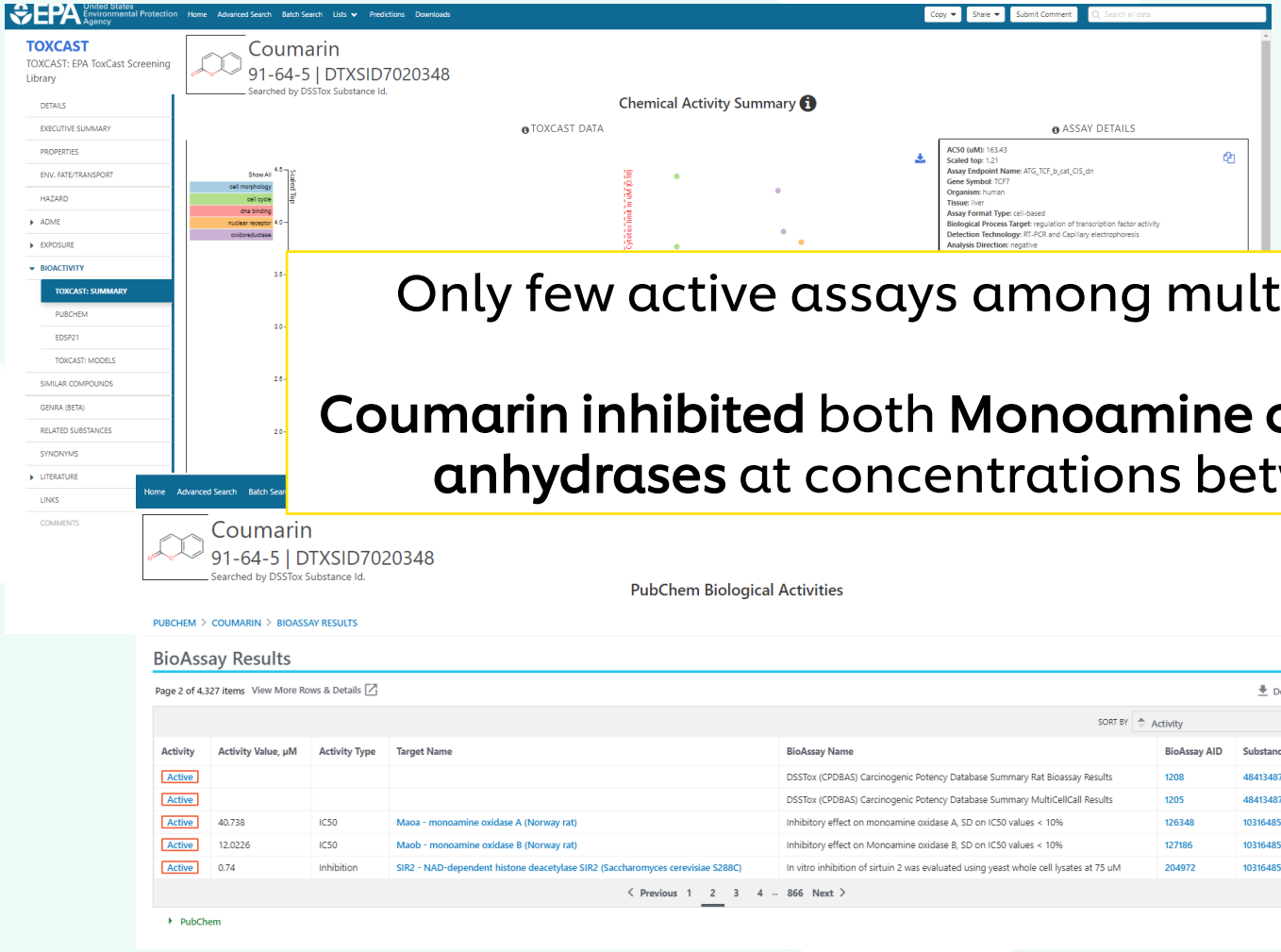
Predicted 22 metabolite pathway (Meteor)

Unilever

NGRA for 0.1% coumarin in face cream: in vitro existing information

EPA iCSS ToxCast Dashboard

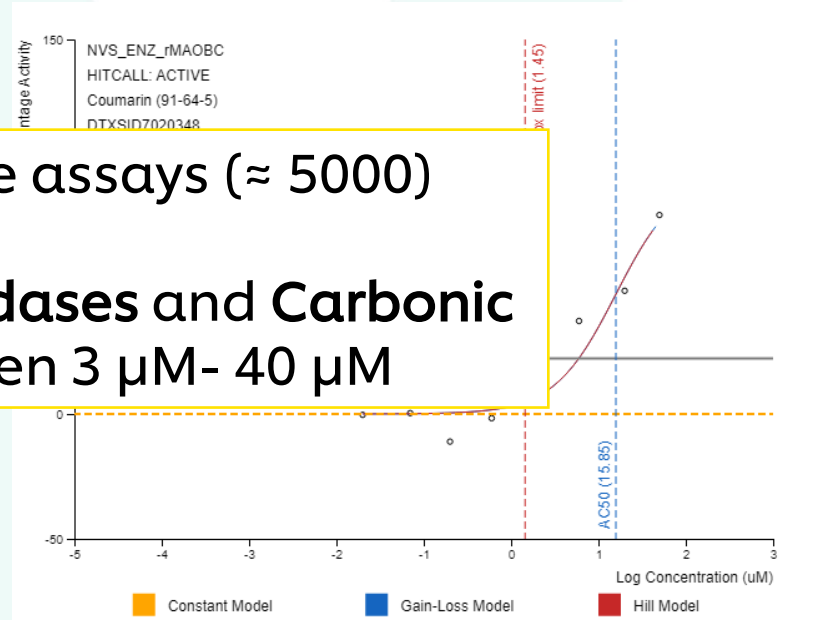
https://comptox.epa.gov/dashboard/dsstoxdb/results?abbreviation=TOXCAST&search=DTXSID7020348#bioactivity



Activity	Activity Value, µM	Activity Type	Target Name	BioAssay Name	BioAssay AID	Substance SID
Active				DSSTox (CPDBAS) Carcinogenic Potency Database Summary Rat Bioassay Results	1208	48413487
Active				DSSTox (CPDBAS) Carcinogenic Potency Database Summary MultiCellCall Results	1205	48413487
Active	40.738	IC50	Maoa - monoamine oxidase A (Norway rat)	Inhibitory effect on monoamine oxidase A, SD on IC50 values < 10%	126348	103164854
Active	12.0226	IC50	Maob - monoamine oxidase B (Norway rat)	Inhibitory effect on Monoamine oxidase B, SD on IC50 values < 10%	127186	103164854
Active	0.74	Inhibition	SIR2 - NAD-dependent histone deacetylase SIR2 (Saccharomyces cerevisiae S288C)	In vitro inhibition of sirtuin 2 was evaluated using yeast whole cell lysates at 75 uM	204972	103164854

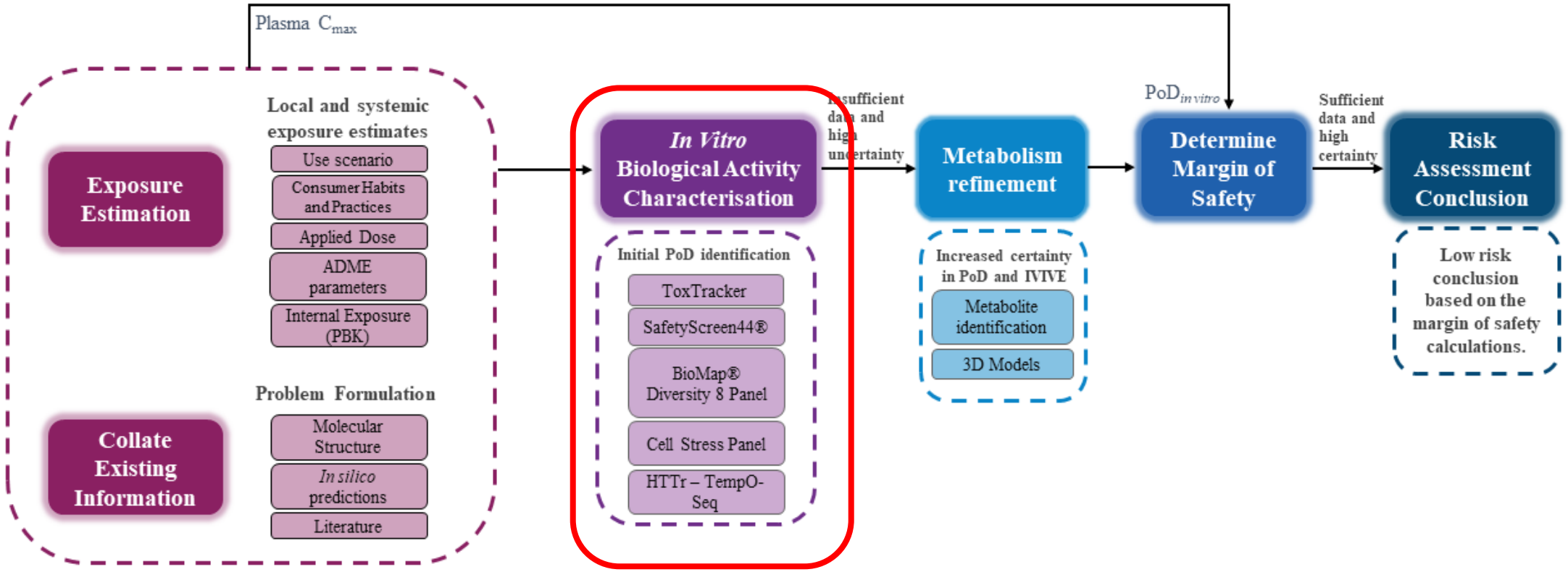
Only few active assays among multiple assays (≈ 5000)

Coumarin inhibited both **Monoamine oxidases** and **Carbonic anhydrases** at concentrations between 3 µM- 40 µM



Winning Model	Model	AIC	RMSE	Top	AC50	Slope
	Constant	81.4	35.91	-	-	-
	Gain-Loss	66.07	7.17	95.63	15.77	1.23
✓	Hill	62.07	7.17	95.63	15.78	1.23

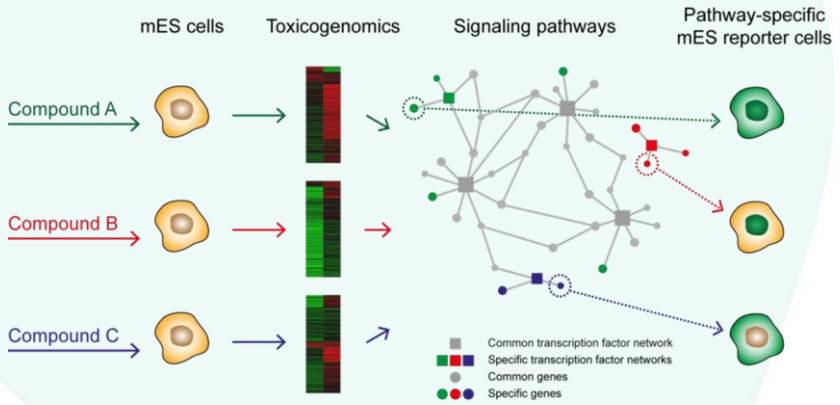
Next-Generation Risk Assessment case study workflow for 0.1% coumarin in face cream

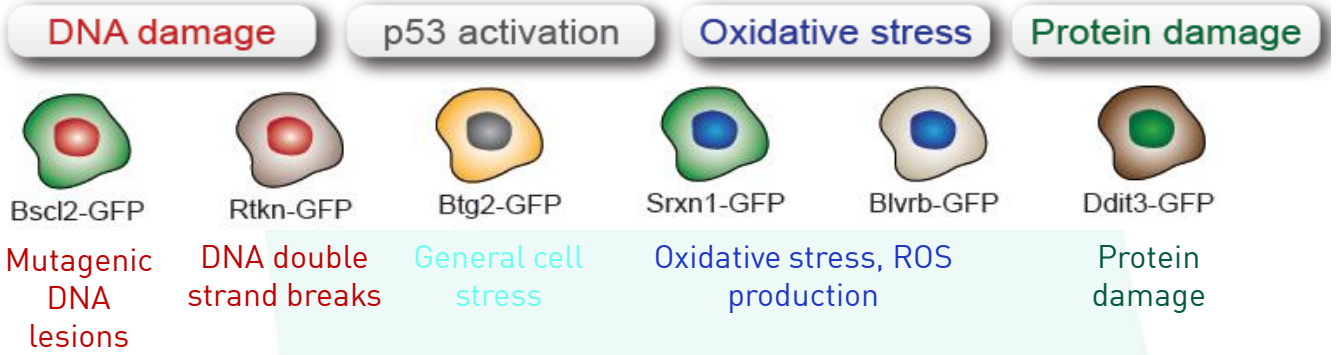


Baltazar *et al.*, (2020) *Tox Sci* (in press)
https://doi.org/10.1093/toxsci/kfaa048

NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: Genotoxicity assessment: ToxTracker

6 GFP reporter mouse embryonic stem (mES) cells





Example of results:

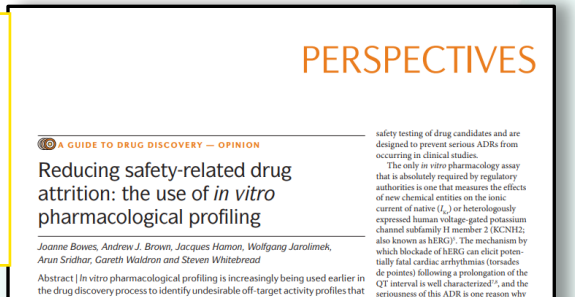
Standard ToxTracker assay +S9					
DNA damage		p53	Ox. stress		UPR
Bscl2	Rtkn	Btg2	Srxn1	Blvrb	Ddit3

Standard ToxTracker assay -S9					
DNA damage		p53	Ox. stress		UPR
Bscl2	Rtkn	Btg2	Srxn1	Blvrb	Ddit3

toxys®

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

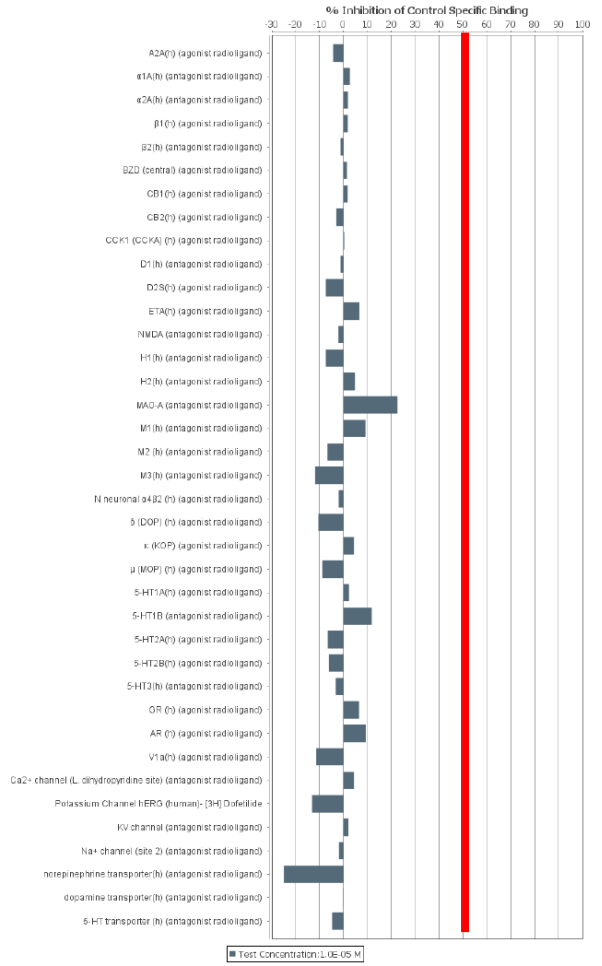
Joanne Bowes, Andrew J. Brown, Jacques Hamon, Wolfgang Jarolimek, Arun Sridhar, Gareth Waldron and Steven Whitebread

Abstract | In vitro pharmacological profiling is increasingly being used earlier in the drug discovery process to identify undesirable off-target activity profiles that

safety testing of drug candidates and are designed to prevent serious ADRs from occurring in clinical studies.

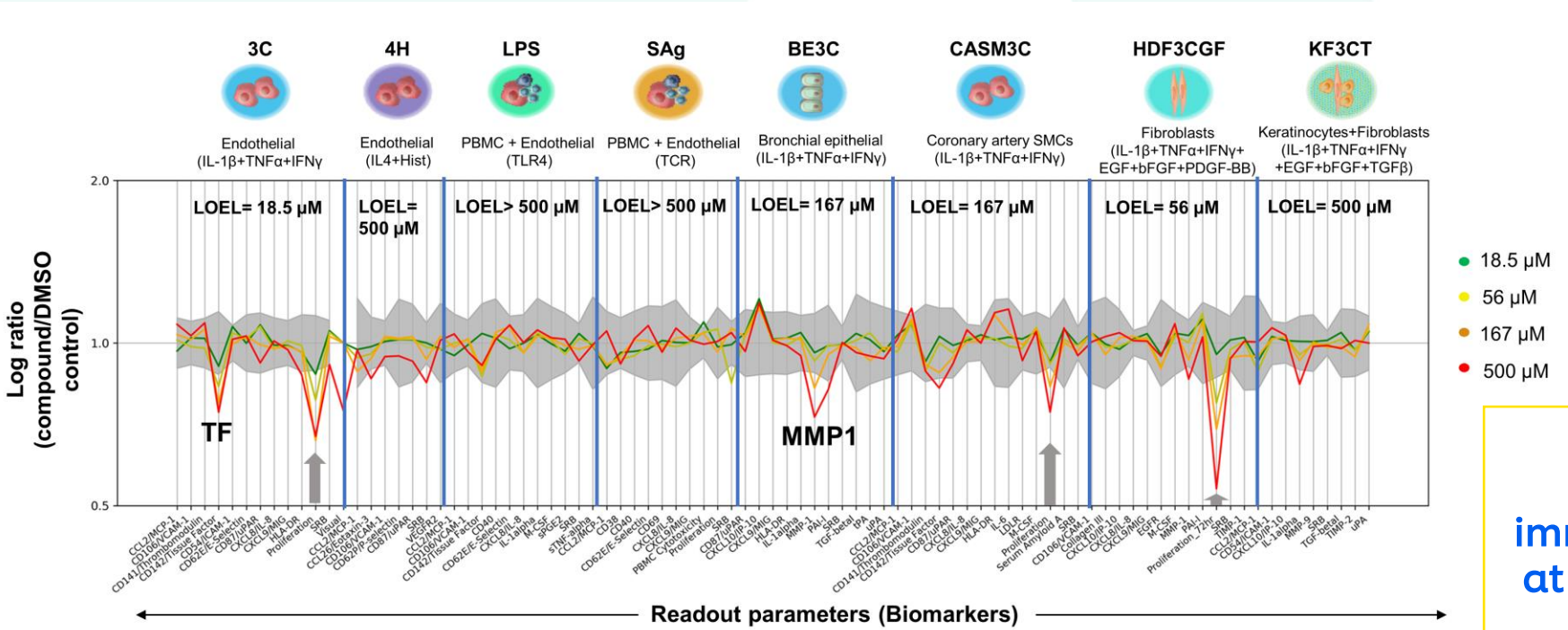
The only *in vitro* pharmacology assay that is absolutely required by regulatory authorities is one that measures the effects of new chemical entities on the ionic current of native (I_{Kr}) or heterologously expressed human voltage-gated potassium channel subfamily H member 2 (KCNH2; also known as hERG)[3]. 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[3,4], and the seriousness of this ADR is one reason why

FAMILY	ASSAY	FORMAT	ITEM #
GPCR			
ADENOSINE	A_{2A}	•	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_{2S}	•	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



NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: Immunomodulatory screening assay: BioMap Diversity 8 Panel

BioMAP systems contain human primary cell types (or combinations) that are stimulated to replicate complex cell and pathway interactions normally found in disease physiology



Data suggested that coumarin has no immunomodulatory effects at relevant concentrations and is not an anti-inflammatory compound

https://www.discoverx.com/services/drug-discovery-development-services/primary-cell-phenotypic-profiling/diversity-plus

NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: In vitro cell stress panel

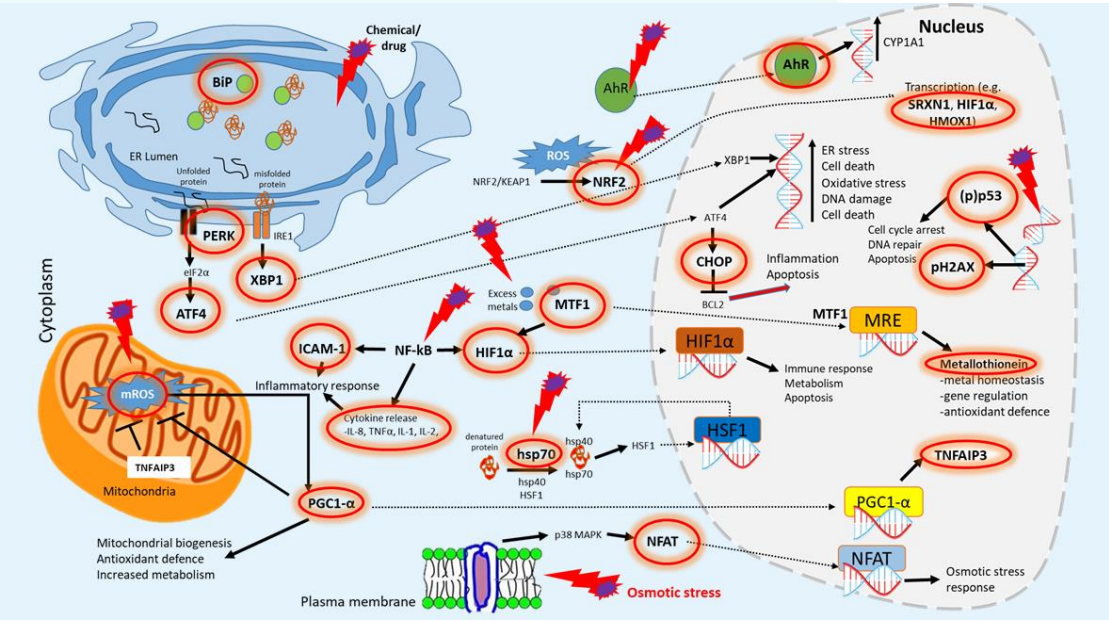


Image kindly provided by Paul Walker (Cyprotex)

OXFORD SOT Society of Toxicology
academic.oup.com/toxsci

TOXICOLOGICAL SCIENCES, 2020, 1–23

doi: 10.1093/toxsci/kfaa054
Advance Access Publication Date: May 6, 2020
Research article

Identifying and Characterizing Stress Pathways of Concern for Consumer Safety in Next-Generation Risk Assessment

Sarah Hatherell,* Maria T. Baltazar,* Joe Reynolds,* Paul L. Carmichael,* Matthew Dent,* Hequn Li,* Stephanie Ryder,† Andrew White,* Paul Walker ,† and Alistair M. Middleton*,1

*Unilever Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, Bedfordshire

36 biomarkers identified that were representative of key stress pathways, mitochondrial toxicity and cell health.

Unilever

NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: In vitro cell stress panel

36 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

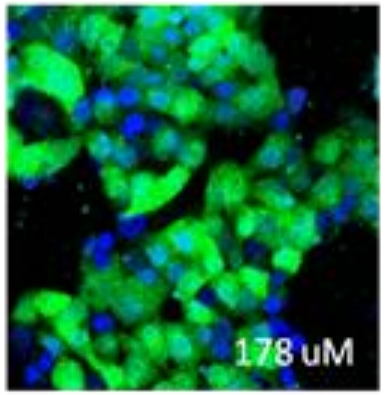
Selection of *in vitro* concentrations based upon realistic human exposures

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

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

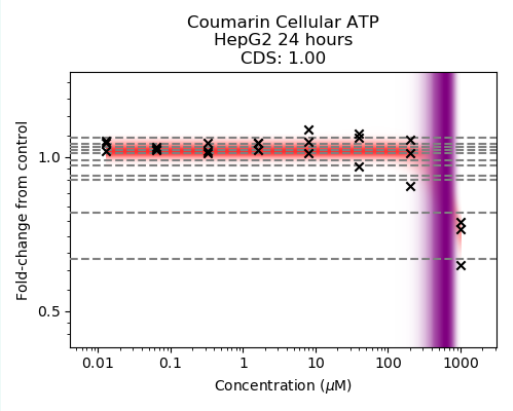
Key
- Exposure scenario adopted for chemical is 'high risk' (from consumer goods perspective).
- Exposure scenario adopted for chemical is 'low risk' (from consumer goods perspective).

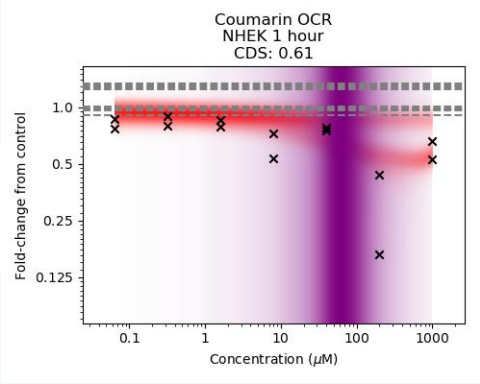
- Mitochondrial Toxicity
- Oxidative Stress
- DNA damage
- Inflammation
- ER Stress
- Metal Stress
- Osmotic Stress
- Heat Shock
- Hypoxia
- Cell Health



*now conducted in HepaRG/NHEK spheroids

NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: In vitro cell stress panel



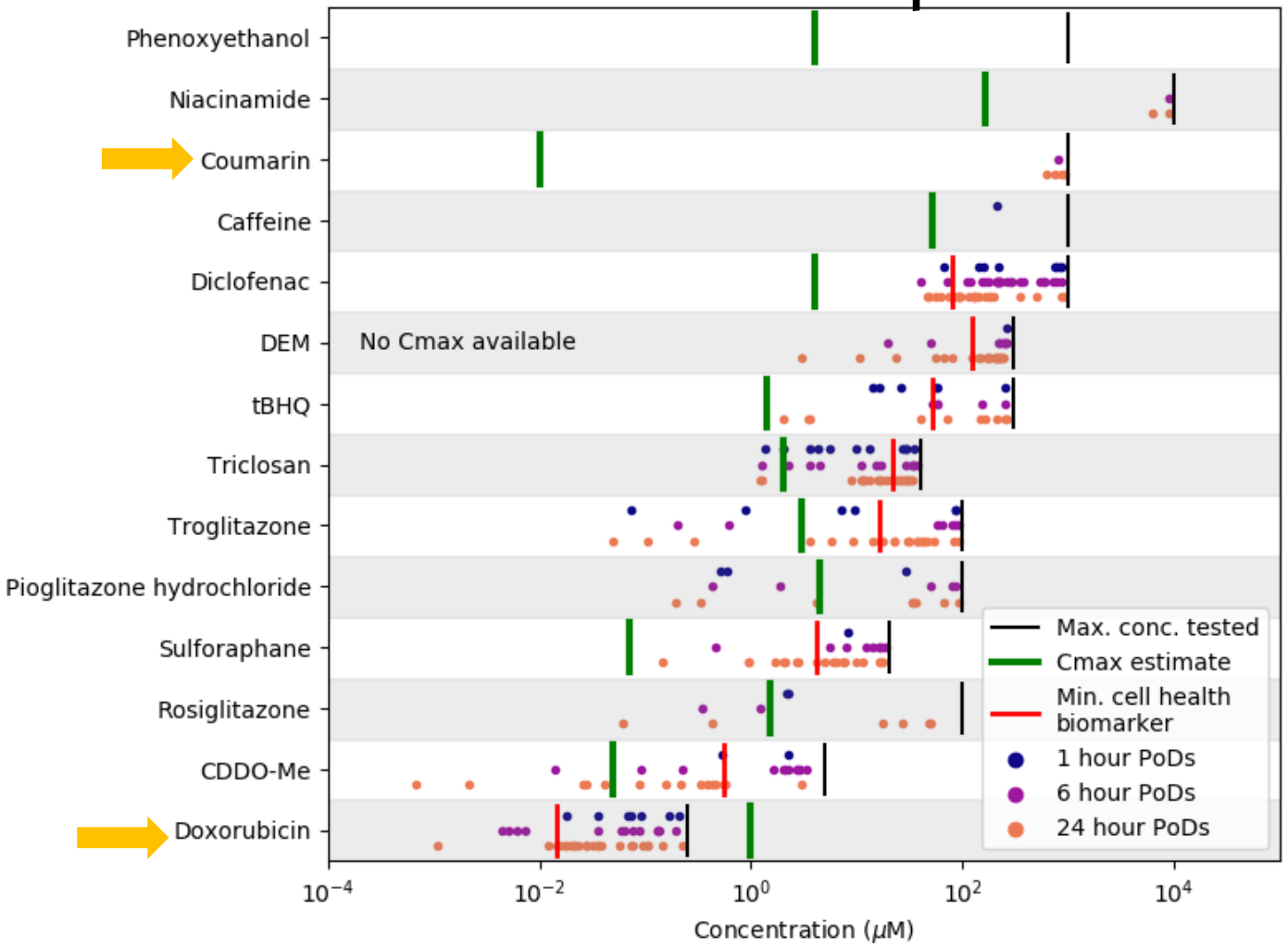


Biomarkers	Cell type	Stress pathway	PoD (μM)	Effect	Concentration dependency score (CDS)
ATP (6h)	HepG2	cell health	794 (363-977)	down	0.98
ATP (24h)			617 (282-891)	down	1
Phospholipidosis (24h)	HepG2	cell health	759 (437-977)	down	0.93
GSH (24h)	HepG2	oxidative stress	851 (301-1000)	up	0.92
IL-8 (24h)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

Hatherell *et al.*, 2020, Identifying and characterizing stress pathways of concern for consumer safety in next generation risk assessment, Tox. Sci. in Press. https://doi.org/10.1093/toxsci/kfaa054

NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: In vitro cell stress panel

Interpreting the data in the context of exposure

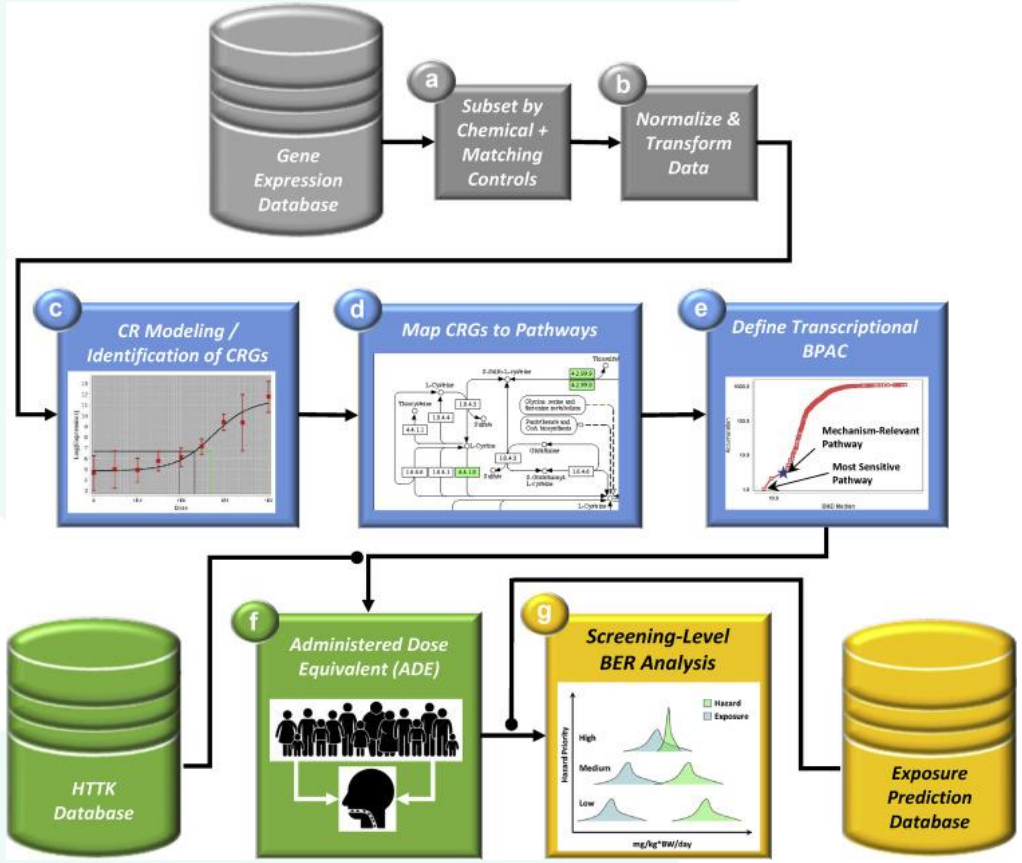


Results:

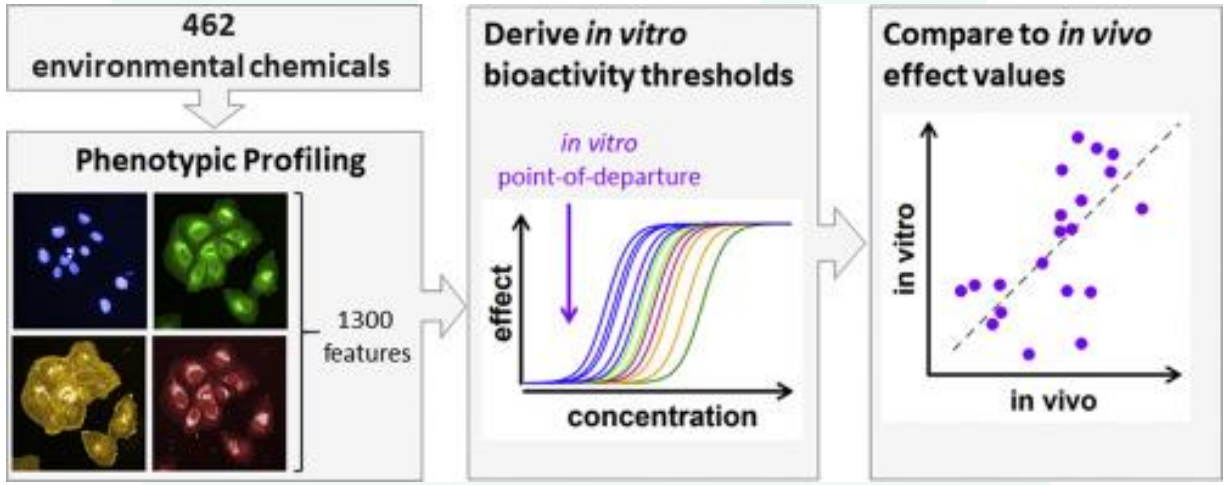
Coumarin not very active in comparison to known "high risk compounds" like doxorubicin

- PoDs shown for HepG2 only

High-throughput transcriptomics and High-throughput phenotypic profiling developed to increase biological coverage



Harrill J et al 2019. Considerations for strategic use of high-throughput transcriptomics chemical screening data in regulatory decisions. Current Opinion in Toxicology 15, 64-75



Nyffeler J et al 2019. Bioactivity screening of environmental chemicals using imaging-based high-throughput phenotypic profiling. *Toxicol Appl Pharmacol.* 2020;389:114876.

Thomas RS et al. The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency. *Toxicol Sci.* 2019;169(2):317-332.

NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: High-Throughput Transcriptomics (HTTr) using TempO-SEQ technology

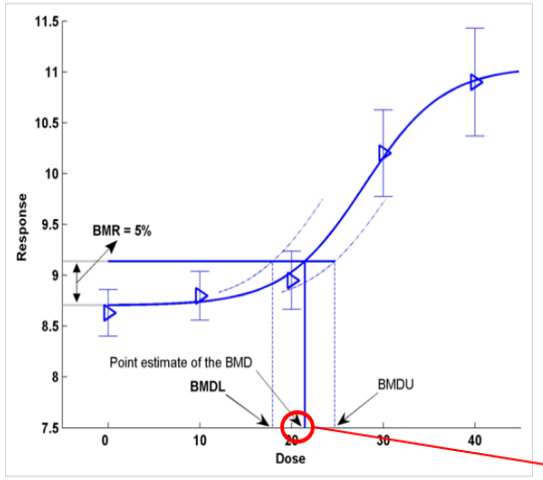
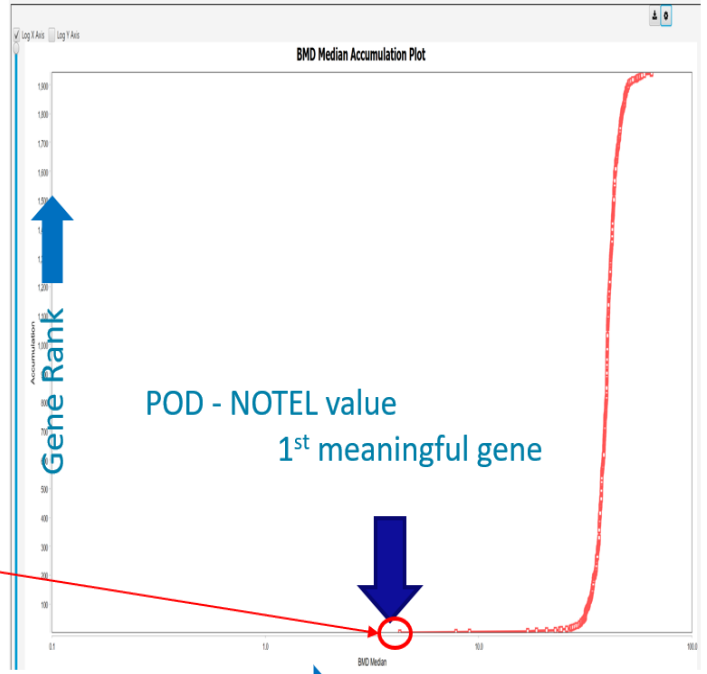
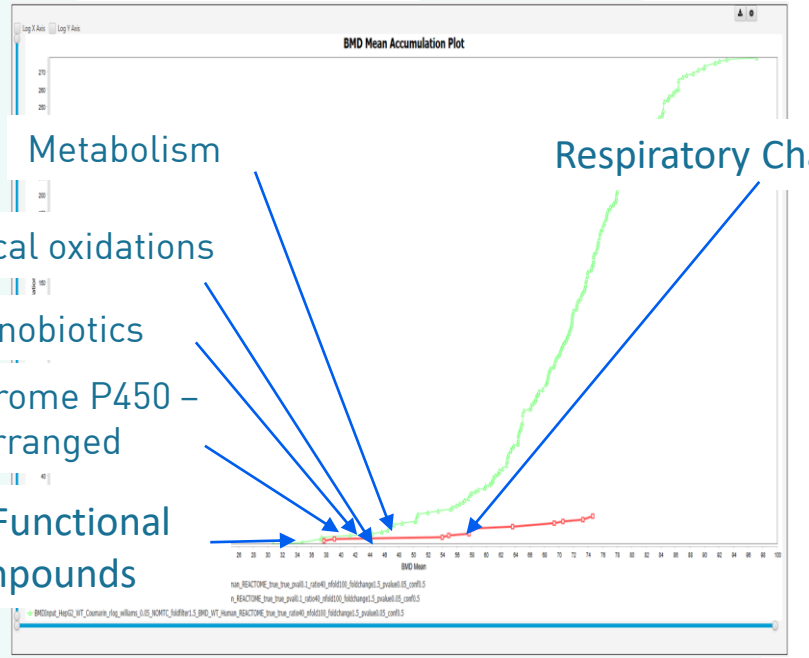


Figure 1 Open in figure viewer | PowerPoint

Key concepts for the BMD approach, illustrated using hypothetical continuous data.





HTTr analysed by BMD Express2

Unilever

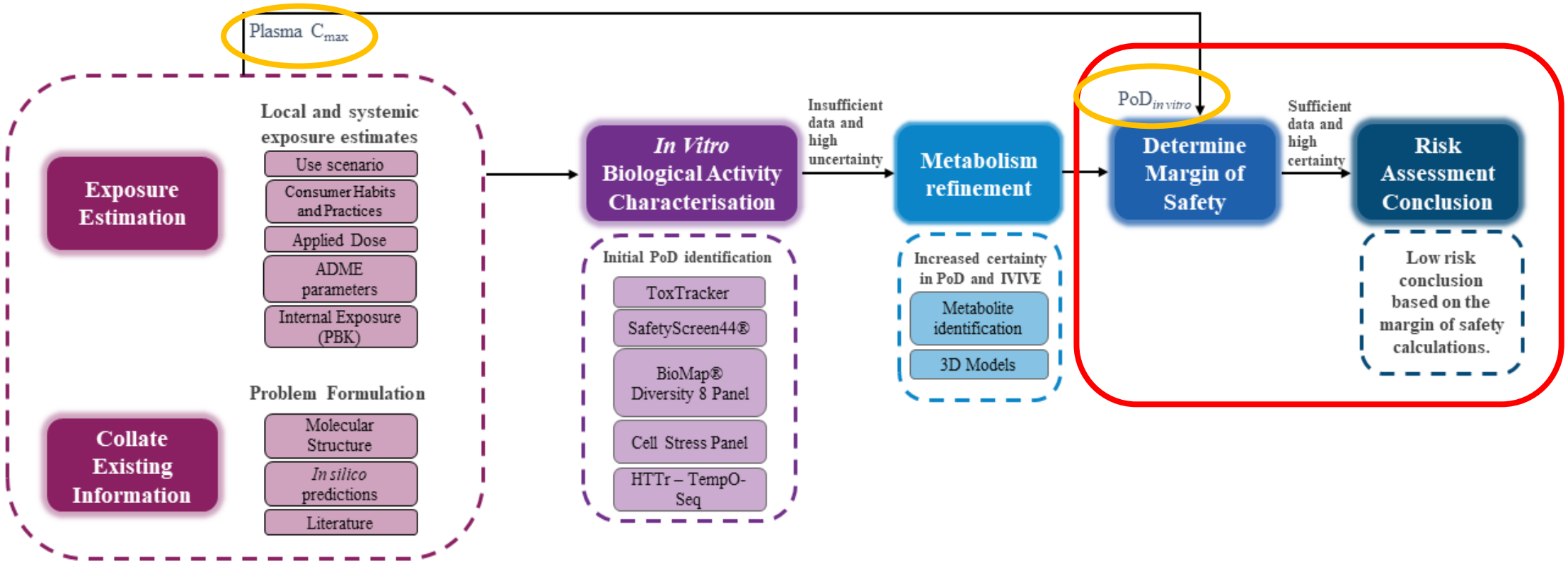
NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: High-Throughput Transcriptomics (HTTr), TempO-SEQ technology

PoD determination

Cell model	HepG2	MCF7	HepaRG 2D
Pathway level tests PoD_T (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_T (µM)	(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

Farmahin, R., Williams, A., Kuo, B. *et al.* Recommended approaches in the application of toxicogenomics to derive points of departure for chemical risk assessment. *Arch Toxicol* **91,** 2045–2065 (2017). https://doi.org/10.1007/s00204-016-1886-5

Next-Generation Risk Assessment case study workflow for 0.1% coumarin in face cream



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

NGRA for 0.1% coumarin in face cream: Determination of Margin of Safety (MoS)

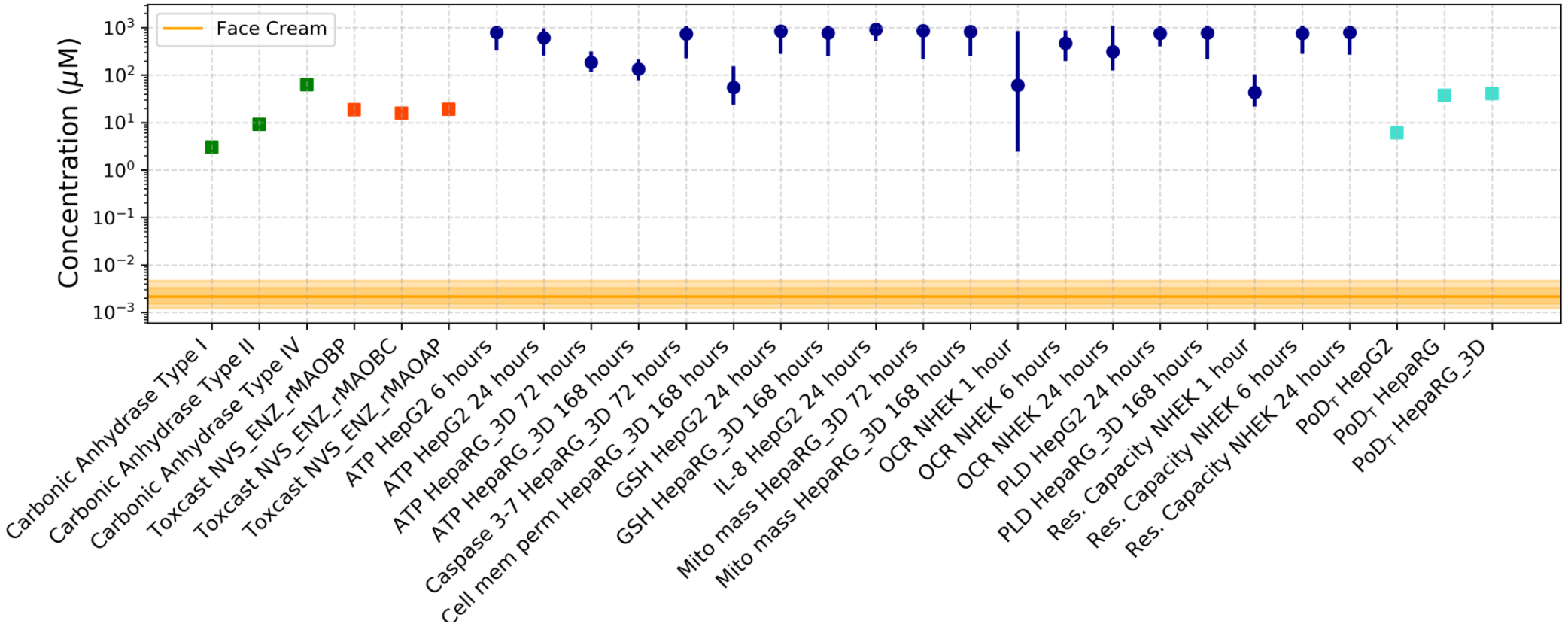
$$MoS = \frac{POD}{Exposure^*}$$

Technology	Cell line/ Enzyme/Biomarker	Face cream Min. 5th percentile MoS	PoD provided as distribution?
Cell stress panel	HepG2 (ATP, 24h)	96738	Yes
Cell stress panel	NHEK (OCR 1h)	1330	Yes
HTTr	HepG2 (24h)	7223	No
HTTr	HepaRG (24h)	8864	No
Toxcast	MAO B (rat brain)	3711	No
PubChem	Carbonic Anhydrase Type I	**706**	No
PubChem	Carbonic Anhydrase Type II	2140	No
PubChem	Carbonic Anhydrase Type VI	14652	No
Cell stress panel	HepaRG_3D (cell mem perm 168h)	9601	Yes
HTTr	HepaRG_3D_24h	9538	No

*Plasma Cmax expressed as distribution

NGRA for 0.1% coumarin in face cream: Risk assessment conclusion

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

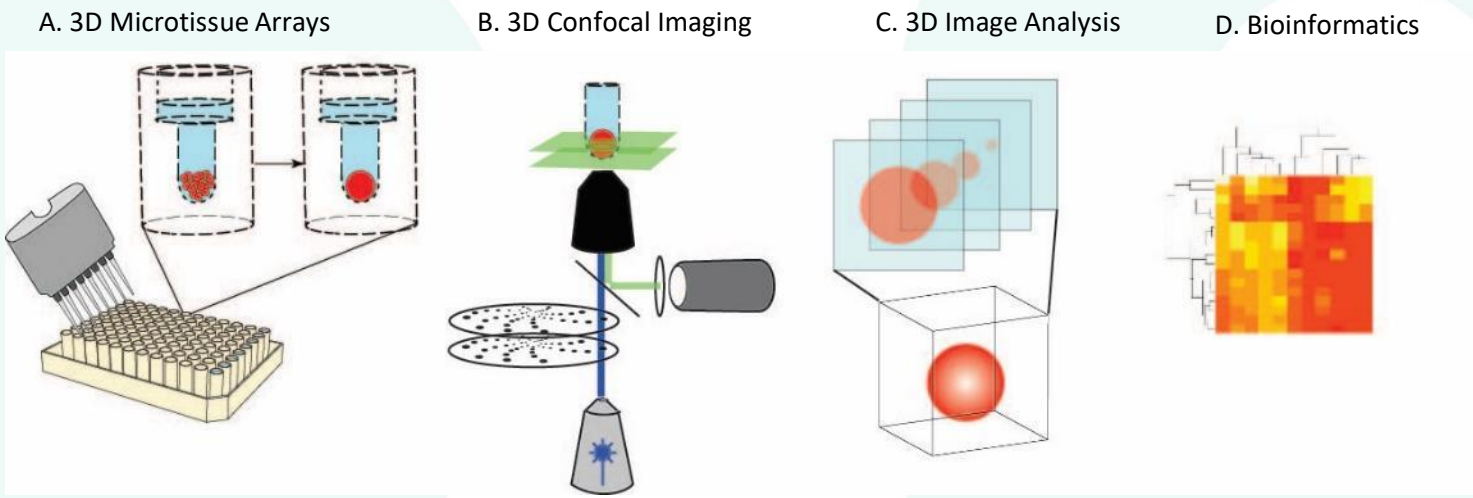


C_{max} expressed as a distribution:

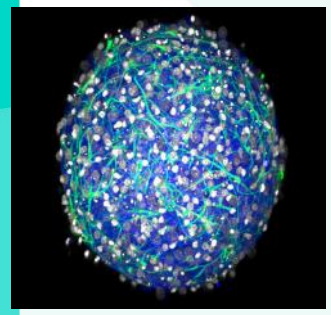
- Red line = median (50th percentile)
- Inner band = 25-75th percentile
- Outer band = 2.5th-97.5th percentile (95th credible interval)

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

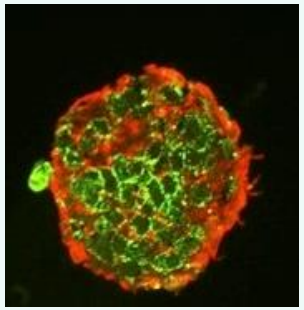
Integrated Morphological and Molecular Responses with Microtissues– Long-term and repeated exposure



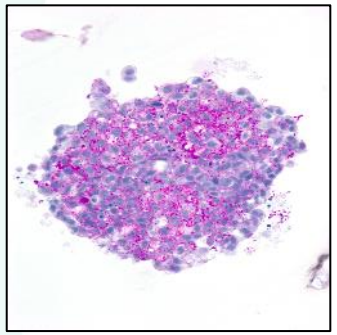
Examples of 3D Microtissues Fabricated at Brown



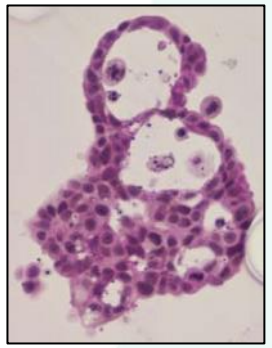
brain



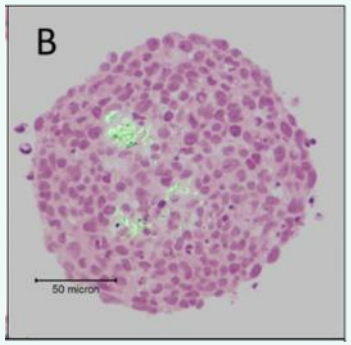
cardiac



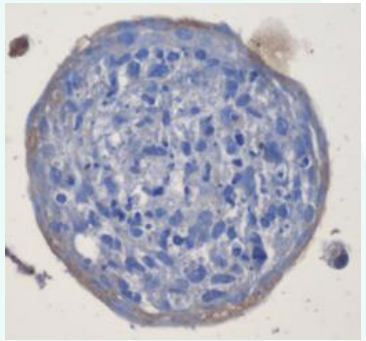
liver



breast

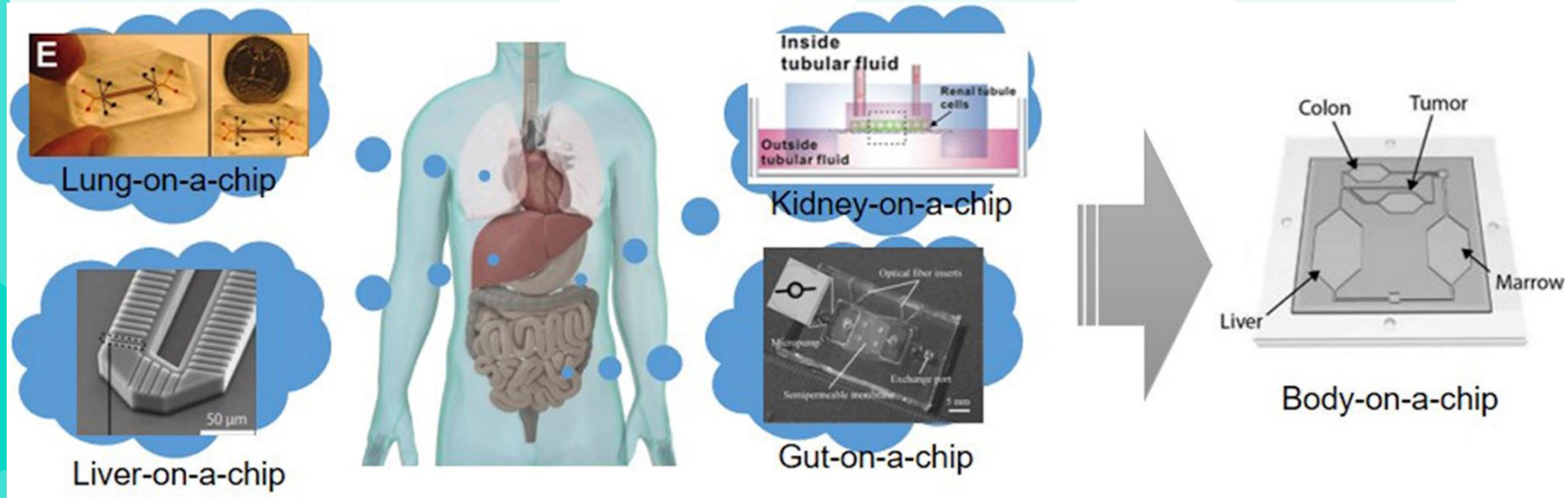


lung



prostate

Advances in organ-on-a-chip engineering…the future of toxicology and personalised medicine?



Kimura et al 2018, Organ/body-on-a-chip based on microfluidic technology for drug discover. Drug Metabolism and Pharmacokinetics Volume 33, Issue 1, Pages 43-48

Concluding remarks

1. Available tools can be integrated to make a safety decision; multidisciplinary team needed!
2. NGRA is a framework of non-standard, bespoke data-generation, driven by the risk assessment questions
3. Need to ensure quality/robustness of the non-standard (non-TG) work and to characterise uncertainty to allow informed decision-making
4. Rethinking MoS/MoE – future evaluation of the approach to infer a low risk space
5. Shortcomings will be addressed by current and future research
6. More research, creativity and examples needed to land this successfully across the community
7. Progress is only possible with a change in mindset (protection not prediction)

Unilever

Acknowledgements

Core Team:

- Maria Baltazar, Alistair Middleton, Tom Cull, Joe Reynolds, Beate Nicol, Mi-Young Lee, Predrag Kukic, Alexis Nathanail, Sophie Cable, Georgia Reynolds, Mona Delagrange, Tom Moxon, Hequn Li,, Mabel Cotter, Jade Houghton, Andy White, Matthew Dent, Paul Carmichael, Sarah Hatherell, Sophie Malcomber, Richard Cubberley, Ruth Pendlington

Extended Team:

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

Any questions?

www.TT21C.org