# Using case studies to increase confidence in Next Generation Risk Assessment


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**Safety Scientist, Unilever, UK**


12th Dec 2021

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# New directions


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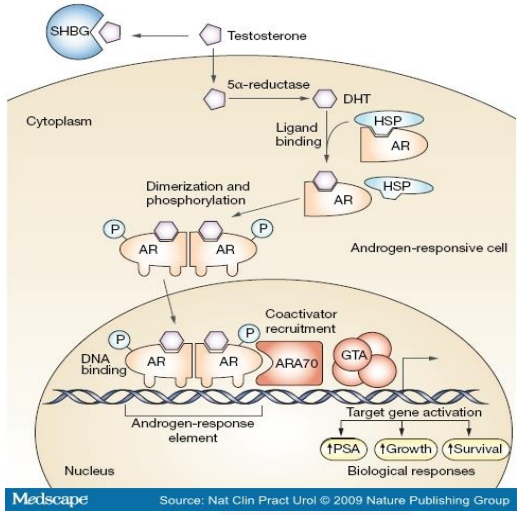
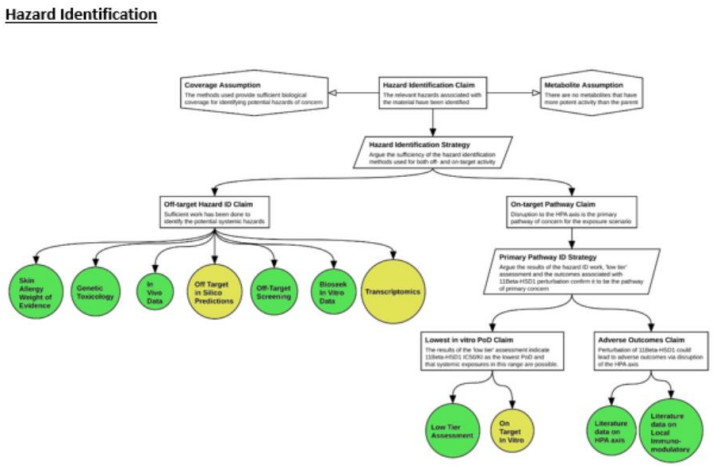
# Next Generation Risk Assessment (NGRA)
## *What is NGRA?*

1. Using new tools and approaches to build a risk assessment to enable decisions to be made

2. An exposure-led risk assessment solution to biological pathway-indicated hazard concerns




Exposure led

Mechanistic

Hypothesis driven

# Chemical safety – India (Using non animal approaches)

1. Animal testing bans in place for cosmetics in many countries across the world
2. Unilever is a part of the Working Group of BIS - updating the Methods of Test for Safety Evaluation of Cosmetics (IS: 4011) as well as Standard for Soaps and Surface Active agents to include non animal approaches

भारतीय मानक ब्यूरो
BUREAU OF INDIAN STANDARDS
मानक भवन, 9 बहादुरशाह ज़फर मार्ग, नई दिल्ली-110002
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI-110002
www.bis.org.in www.standardsbis.in

IS 4011 : 2018

*Indian Standard*

METHODS OF TEST FOR
SAFETY EVALUATION OF COSMETICS

**ANNEX B**

[*Table* 1, *Sl No.* (iii)]

**ALTERNATE METHODS FOR SAFETY TESTING**

*(Source Reference — OECD Guidelines, EURL ECVAM Recommendations)*

# Can we use a new ingredient safely?

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

# EU scientific committee on consumer safety (SCCS)



https://ec.europa.eu/health/sites/default/files/scientific_committees/consumer_safety/docs/sccs_o_250.pdf

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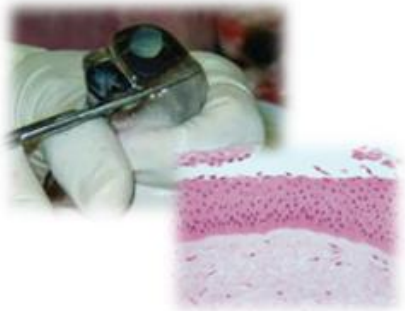
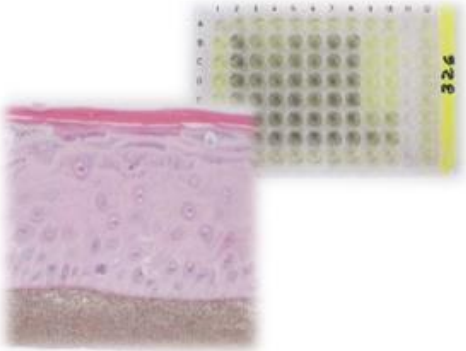
# OECD tests that don't use animals: used for many end-points

OECD TG438



OECD TG437

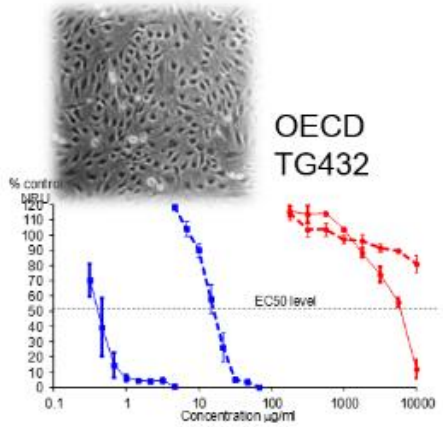
Eye Irritation



OECD TG430/431

OECD TG439

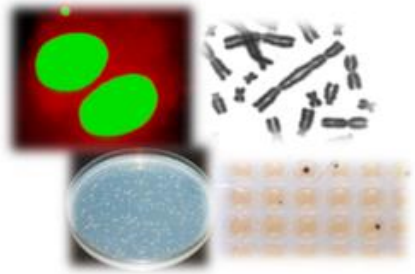
Skin Corrosion/Irritation




Phototoxicity

OECD TG487

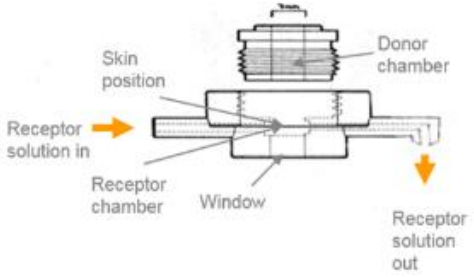
OECD TG473



OECD TG471

OECD TG476

Genotoxicity

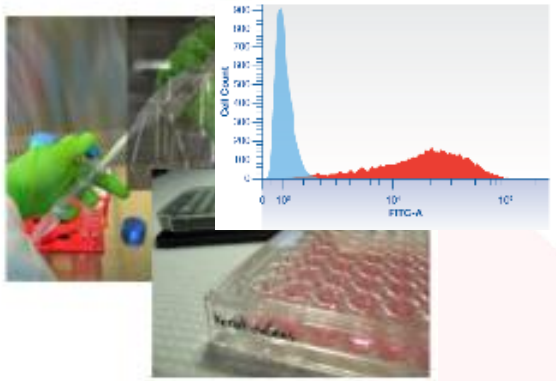



OECD TG428

Skin Penetration

OECD TG442C

OECD TG442E

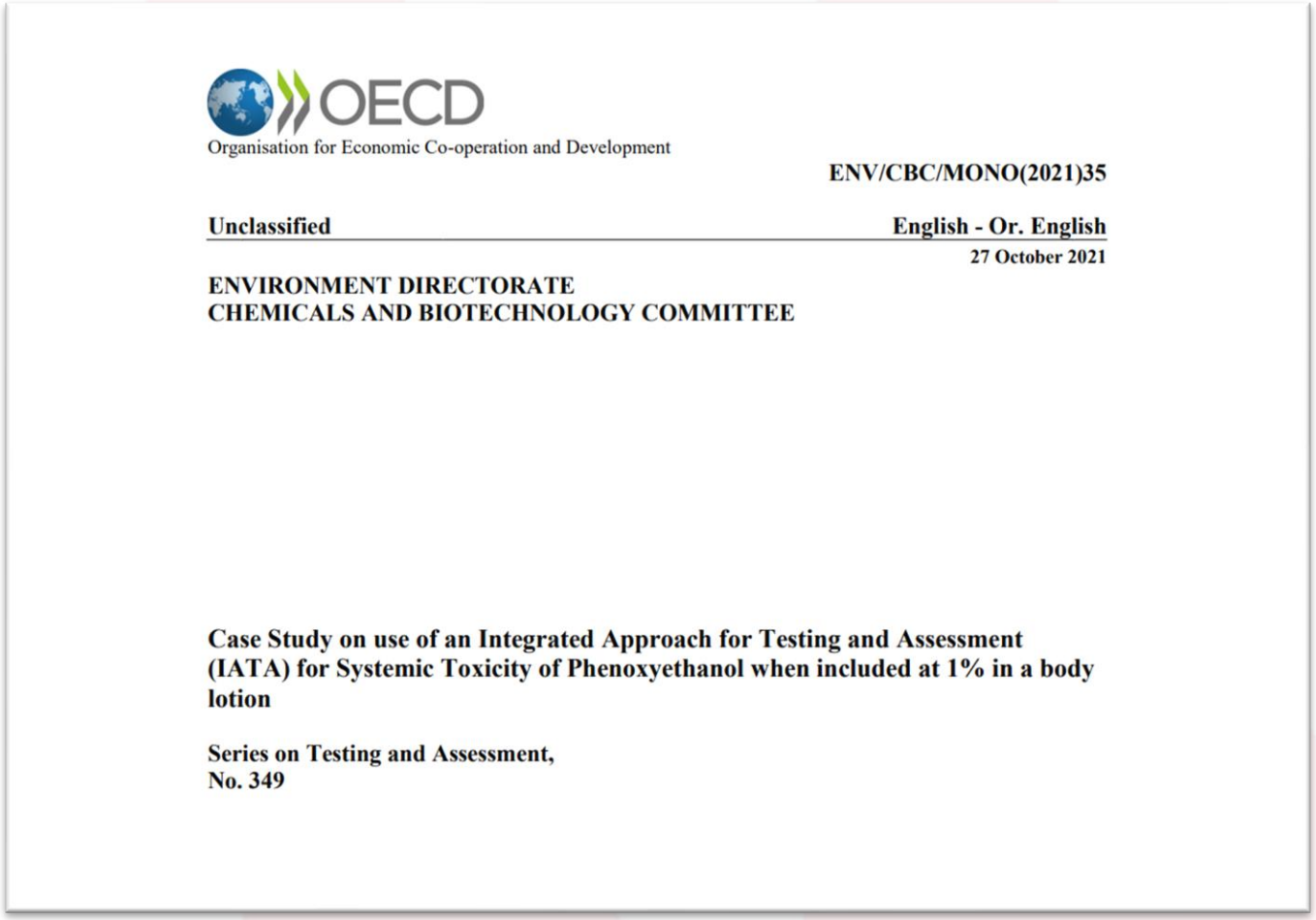


OECD TG442D

Skin Sensitisation

# Outline

1. Case study background: assuring the safety of 1% phenoxyethanol in body lotion
2. Methods used
3. Results
4. Conclusion



OECD

Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2021)35

Unclassified — English - Or. English

27 October 2021

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

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

Series on Testing and Assessment,
No. 349

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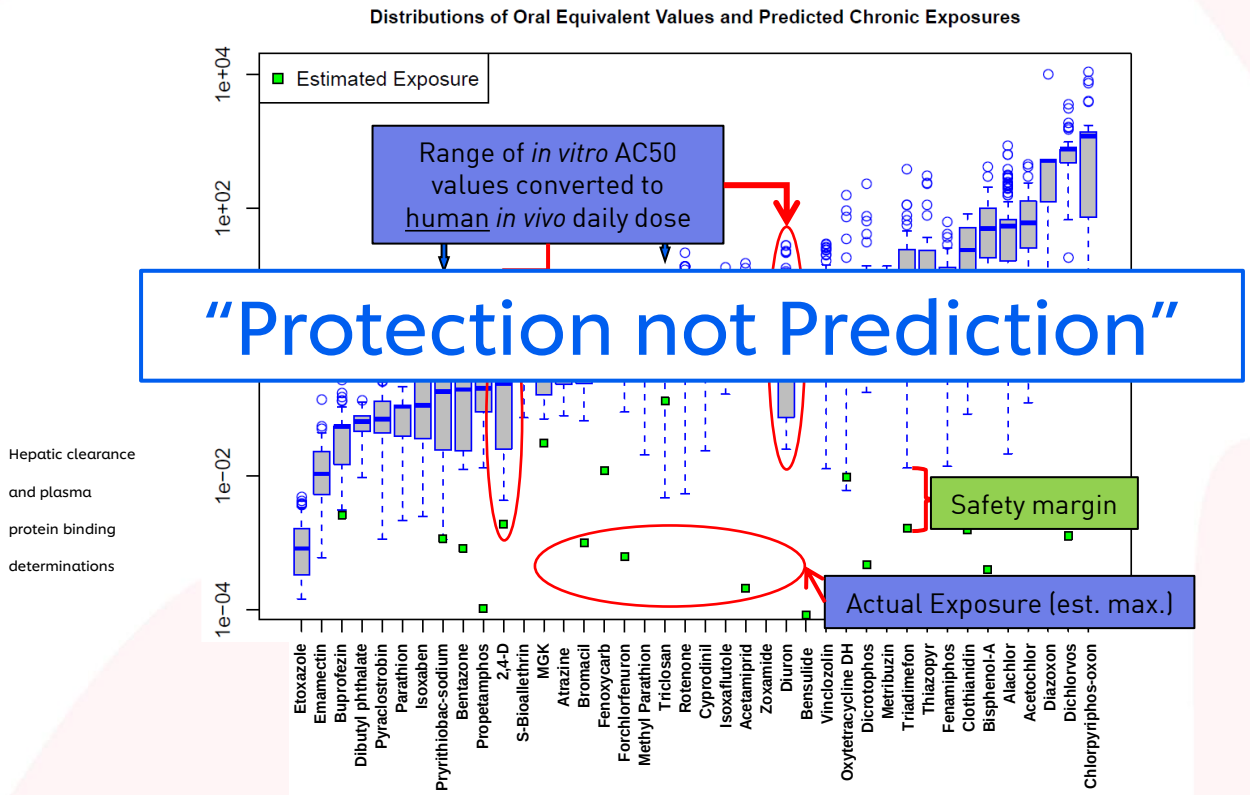
# Context of Case study: *In Vitro* Bioactivity vs Bioavailability

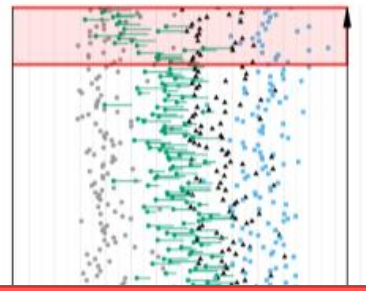



Slide from Dr Rusty Thomas, EPA, with thanks

Rotroff, *et al.* Tox.Sci 2010

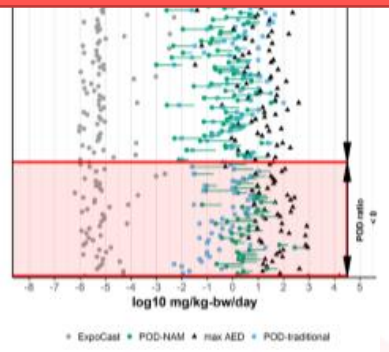
# EPA, NTP, HC, A*STAR, ECHA, EFSA, JRC, RIVM…

APCRA
ACCELERATING THE PACE OF CHEMICAL RISK ASSESSMENT



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

**Can we apply a similar bioactivity: exposure comparison not for the purposes of prioritization but for safety assessment?**


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

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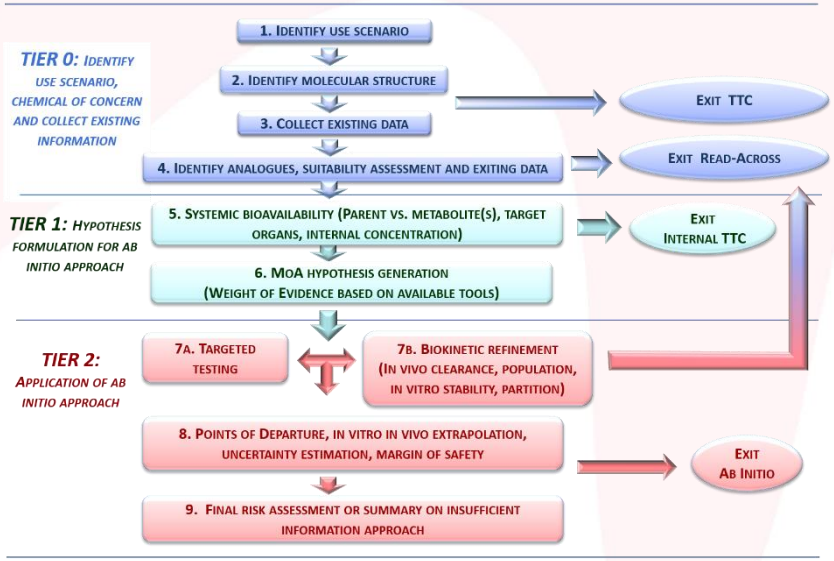
# Guiding principles




SEURAT-1 Framework (Gocht *et al* 2014)


Computational Toxicology 7 (2018) 20–26
Contents lists available at ScienceDirect
Computational Toxicology
journal homepage: www.elsevier.com/locate/comtox
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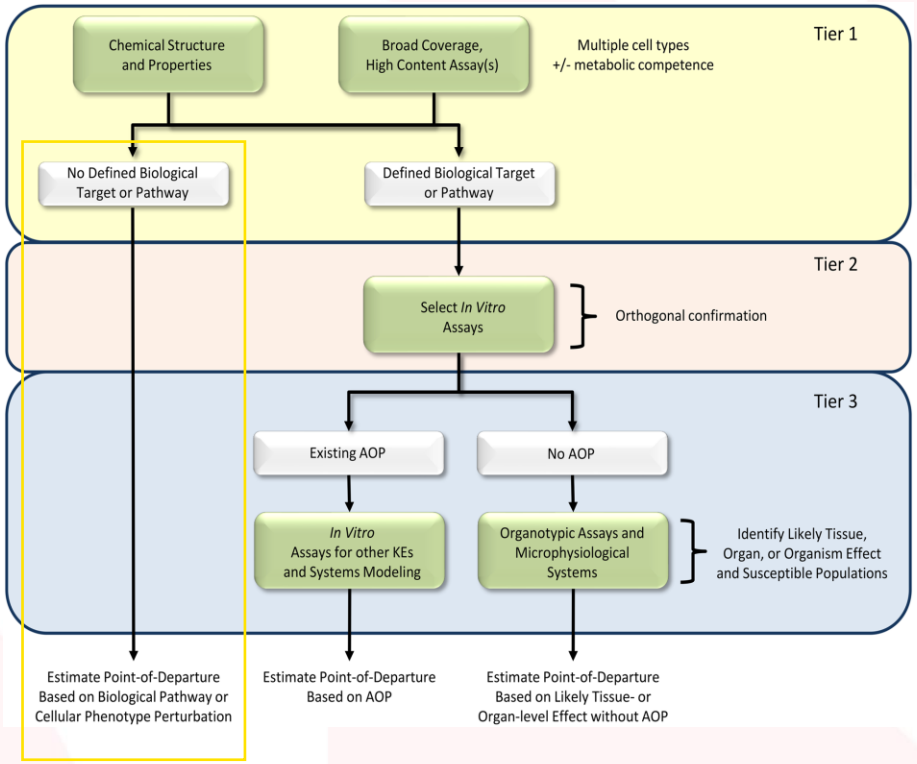
Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients

Matthew Dent[a,*], Renata Teixeira Amaral[b], Pedro Amores Da Silva[b], Jay Ansell[c], Fanny Boisleve[d], Masato Hatao[e], Akihiko Hirose[f], Yutaka Kasai[g], Petra Kern[h], Reinhard Kreiling[i], Stanley Milstein[j], Beta Montemayor[k], Julcemara Oliveira[l], Andrea Richarz[m], Rob Taalman[n], Eric Vaillancourt[o], Rajeshwar Verma[j], Nashira Vieira O'Reilly Cabral Posada[l], Craig Weiss[p], Hajime Kojima[f]

ICCR Principles (Dent *et al* 2018)

SEURAT-1

OXFORD UNIVERSITY PRESS




*Toxicological Sciences*, Volume 169, Issue 2, June 2019, Pages 317–332, https://doi.org/10.1093/toxsci/kfz058



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# ICCR Nine principles of NGRA

## 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 transparent and documented

ICCR International Cooperation on Cosmetics Regulation

Computational Toxicology 7 (2018) 20–26

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[c] US Personal Care Products Council (PCPC), 1620 I St. NW, Suite 1200, Washington, D.C. 20036, USA
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[e] Japan Cosmetic Industry Association (JCIA), Metro City Kamiyacho 6F, 5-1-5, Toranomon, Minato-ku, Tokyo 105-0001 Japan
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[g] Kao Corporation, External Relations & Government Affairs 2-1-3, Bunka, Sumida-Ku, Tokyo 131-8501 Japan
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[k] Cosmetics Alliance Canada, 420 Britannia Road East Suite 102, Mississauga, ON L4Z 3L5, Canada
[l] Brazilian Health Regulatory Agency (ANVISA), Gerência de Produtos de Higiene, Perfumes, Cosméticos e Saneantes, SIA Trecho 5, lote 200, Area Especial 57 – CEP 71205-050, Brazil
[m] European Commission, Joint Research Centre (JRC), Directorate for Health, Consumers and Reference Materials, Chemical Safety and Alternative Methods Unit, Via E. Fermi 2749, 21027 Ispra, VA, Italy
[n] Cosmetics Europe, Avenue Herrmann-Debroux 40, 1160 Auderghem, Belgium
[o] Health Canada (HC), Consumer Product Safety Directorate, Healthy Environments and Consumer Safety Branch, 269 Laurier Ave. W., Ottawa, ON K1A 0K9, Canada
[p] Independent Cosmetic Manufacturing and Distributors (ICMAD), 21925 Field Parkway, Suite 2015, Deer Park, IL 60010, USA

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# Existing data

1. Data for 1,646 assays found in the PubChem database, 3 non-animal assays positive:

- Human retinoid X receptor (RXR) agonism
- Aryl hydrocarbon receptor (AhR) activation
- Human SUMO peptidase NEDD8 specific (SENP8) inhibition
- loss of function results in defective cell cycle progression

2. Data for 785 ToxCast/Tox21 assays (1 potential positive)

- Trans-activation of retinoic acid receptors (RARa, RARb, RARg, RXRa, RXRb and RXRg) and cis-activation of DR5 response elements by RAR/RXR negative
- AhR assays negative (one of these was the same assay reported above using a different analysis method)
- Positive hit from one of the BioMap Diversity 8 assays

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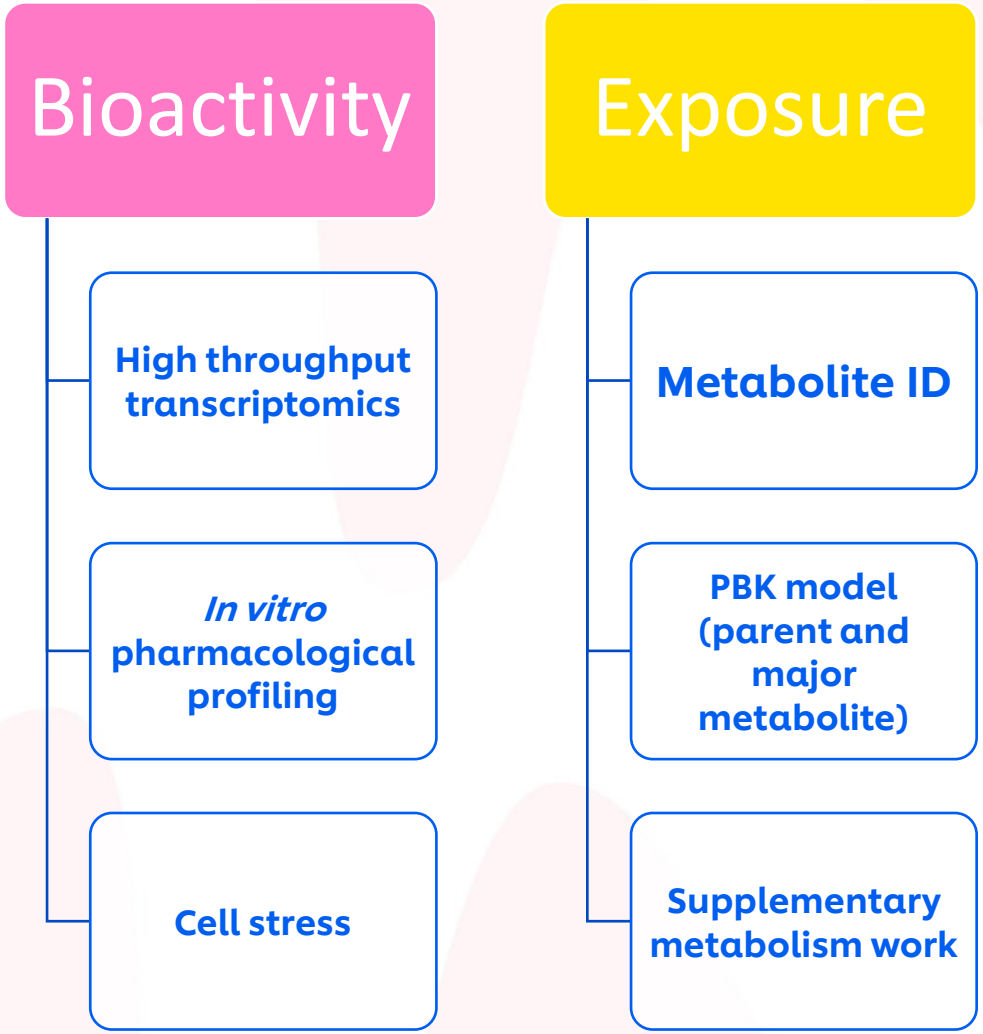
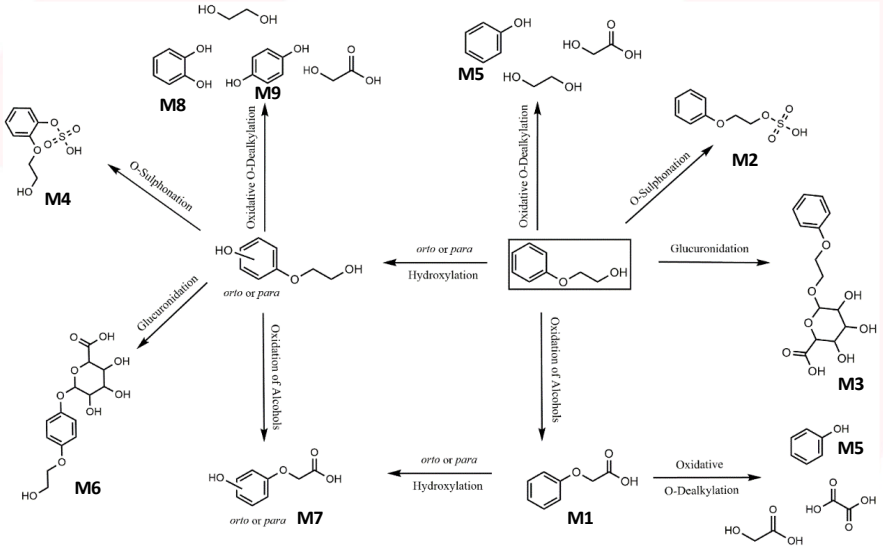
# New data generation and predictions

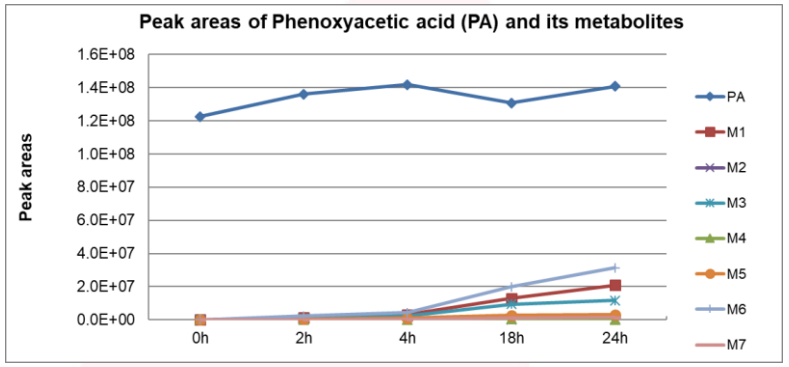


# Exposure assessment

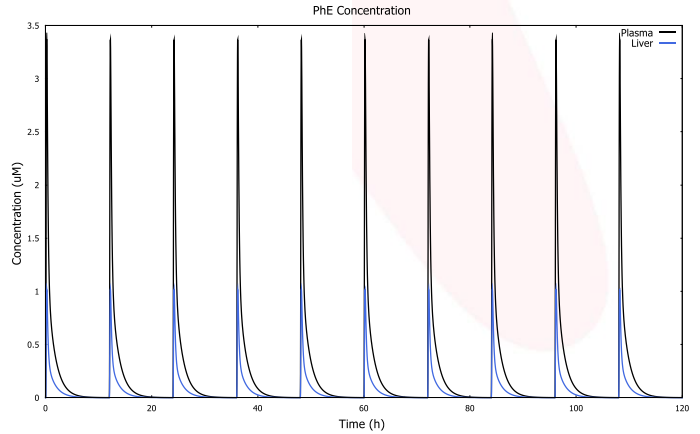
## *In silico* predictions

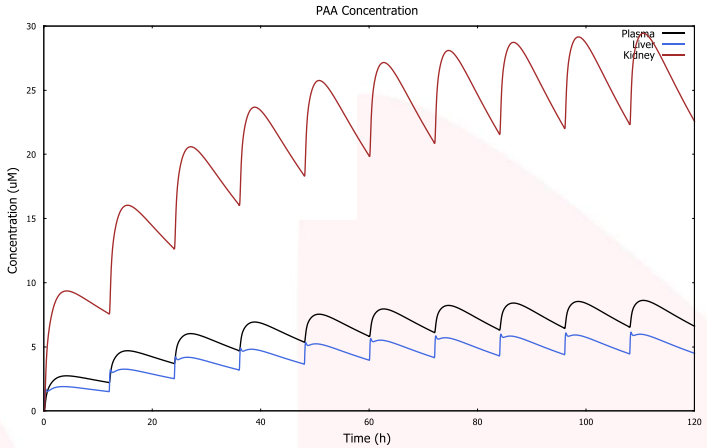


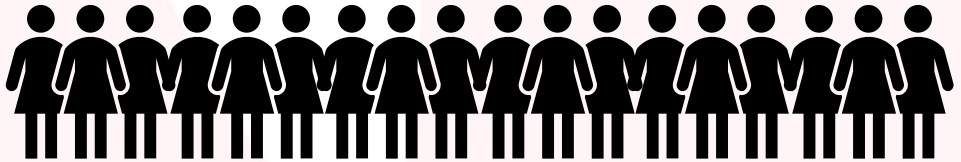

## *In vitro* confirmation




## PBK modelling (parent and metabolite)



# Population PBK modelling output

| | Blood | Blood | Blood | Blood | Kidney | Kidney |
|---|---|---|---|---|---|---|
| | PhE $C_{max}$ | PhE $AUC_{24}$ | PAA $C_{max}$ | PAA $AUC_{24}$ | PAA $C_{max}$ | PAA $AUC_{24}$ |
| | µM | µmol*h/L | µM | µmol*h/L | µM | µmol*h/L |
| Average | 3.7 | 7.3 | 10.5 | 230 | 36 | 789 |
| SD | 1.4 | 4.2 | 4.9 | 115 | 17 | 401 |
| 5th %ile | 1.8 | 3.3 | 4.5 | 93 | 15 | 312 |
| Median | 3.6 | 6.2 | 9.3 | 206 | 32 | 699 |
| 95th %ile | 6.2 | 15 | 20 | 453 | 69 | 1569 |

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# *In silico* tools to identify possible MoA

- Derek Nexus (v 5.0.2 Lhasa Ltd)
  - inactive (negative) in the Ames assay
- OECD QSAR Toolbox v. 4.1
  - *in vivo* mutagenicity (micronucleus) in rodents: alert for H-acceptor-path3-H-acceptor
- CERAPP and CoMPARA
  - no binding predicted
- COSMOS profilers (https://knimewebportal.cosmostox.eu/com.knime.enterprise.server/#login /)
  - potential binding to Thyroid Hormone Receptor (THR)
- MIE Atlas (Allen et al. (2018) Tox Sciences doi: 10.1093/toxsci/kfy144)
  - no alerts

ToxCast: Models

ToxCast Model Predictions

Download ToxCast Model Predictions

| Model | Receptor | Agonist | Antagonist | Binding |
|---|---|---|---|---|
| ToxCast Pathway Model (AUC) | Androgen | 0.00 | 0.00 | - |
| ToxCast Pathway Model (AUC) | Estrogen | 0.00 | 0.00 | - |
| COMPARA (Consensus) | Androgen | Inactive | Inactive | Inactive |
| CERAPP Potency Level (From Literature) | Estrogen | Inactive (Inactive) | Inactive (Inactive) | Inactive (Inactive) |
| CERAPP Potency Level (Consensus) | Estrogen | Inactive (Inactive) | Inactive (Inactive) | Inactive (Inactive) |

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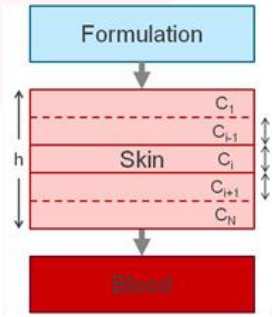
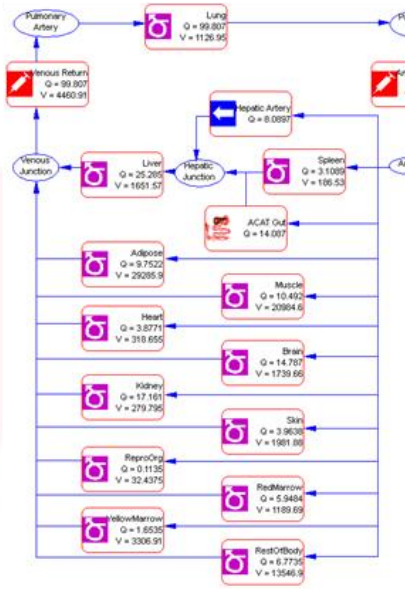
# Bioactivity assays

**In Vitro Assays:**

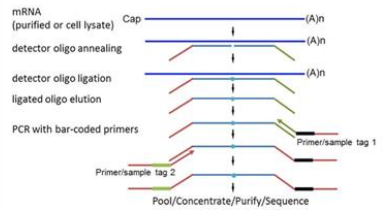
Kinetic Solubility
Thermodynamic Solubility
Metabolic Stability
-Human Hepatocytes
-Human CYP450 Isoforms
-Human Hepatic Microsomes
Stability in Human Plasma
Plasma Protein Binding
Partitioning in Human Blood

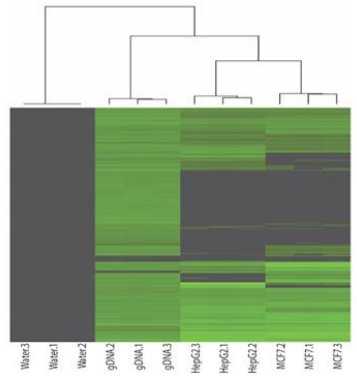



## PBPK Modelling

BioSpyder cyprotex




eurofins | Cerep **SafetyScreen44™ Panel**

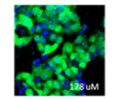
| FAMILY | ASSAY | FORMAT | ITEM # |
|---|---|---|---|
| ■ GPCR | | | |
| ADENOSINE | $A_{2A}$ | • | 0004 |
| ADRENERGIC | alpha$_{1A}$ | | 2338 |
| | alpha$_{2A}$ | | 0013 |
| | beta$_1$ | • | 0018 |
| | beta$_2$ | • | 0020 |
| CANNABINOID | $CB_1$ | • | 0036 |
| | $CB_2$ | • | 0037 |
| CHOLECYSTOKININ | CCK$_2$ (CCK$_B$) | • | 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-$HT_{1A}$ | • | 0131 |
| | 5-$HT_{1B}$ | | 0132 |
| | 5-$HT_{2A}$ | • | 0471 |
| | 5-$HT_{2B}$ | • | 1333 |
| VASOPRESSIN | $V_{1a}$ | • | 0159 |
| ■ TRANSPORTERS | | | |
| DOPAMINE | dopamine transporter | | 0052 |

| FAMILY | ASSAY | FORMAT | ITEM # |
|---|---|---|---|
| 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-$HT_3$ | | 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 |

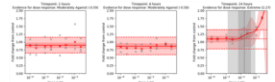
## CELL STRESS PANEL

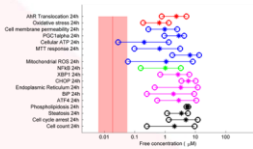
cyprotex

- Range of biomarkers covering ~10 cell stress pathways:




**Mitochondrial Toxicity:** MitoSOX, PGC1α, MMP, ATP, Glu/Gal
**Oxidative Stress:** GSH, ROS, SRXN1, NRF2
**DNA damage:** pH2AX, p53
**Inflammation:** TNFAIP3, ICAM1, NFkB p65, IL-1β, IL-8, HMGB1
**ER Stress:** PERK, ATF4, CHOP, XBP1, BiP, ER Tracker
**Metal Stress:** MTF-1, Metallothionein
**Osmotic Stress** (NFAT5); **Heat Shock** (HSP70); **Hypoxia** (HIF1α)
**Cell Health**: LDH, Phospholipidosis, Steatosis, pHrodo indicator, apoptosis (caspase-3/7) & necrosis (ToPro-3)





| | 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) |

**BioMap Diversity 8 Panel (ToxCast)**

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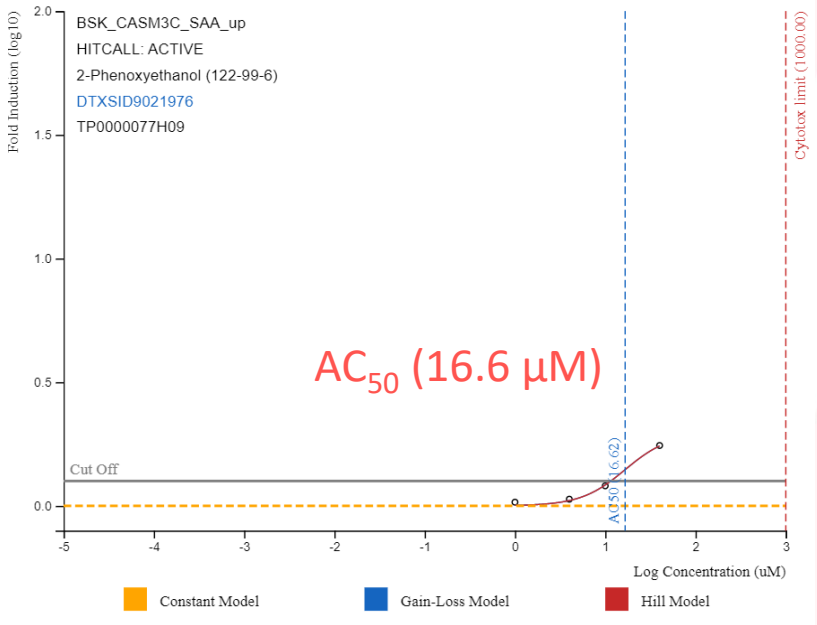
# BioMap Diversity 8 Panel (ToxCast)

Houck, K. A. et al. (2009) Journal of Biomolecular Screening. 14(9), 1054–1066.

doi:10.1

| | 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) |




$AC_{50}$ (16.6 µM)

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

**No significant change in any other cells or markers (including IL8 or IL6)**

# *In Vitro* Pharmacological Profiling

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

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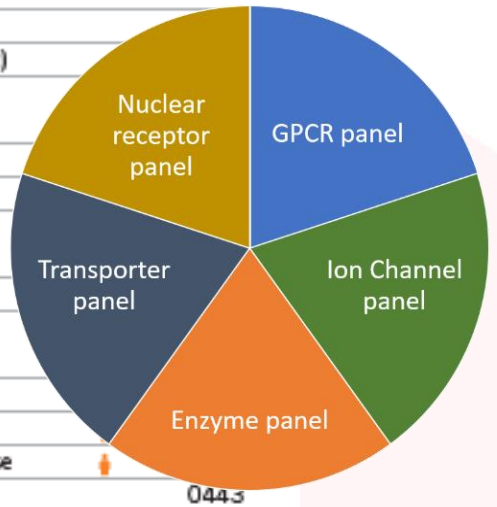
| FAMILY | ASSAY | FORMAT | ITEM # |
|---|---|---|---|
| **GPCR** | | | |
| ADENOSINE | $A_{2A}$ | • | 0004 |
| ADRENERGIC | $alpha_{1A}$ | | 2338 |
| | $alpha_{2A}$ | | 0013 |
| | $beta_1$ | • | 0018 |
| | $beta_2$ | • | 0020 |
| CANNABINOID | $CB_1$ | • | 0036 |
| | $CB_2$ | • | 0037 |
| CHOLECYSTOKININ | $CCK_1$ ($CCK_A$) | • | 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-$HT_{1A}$ | • | 0131 |
| | 5-$HT_{1B}$ | | 0132 |
| | 5-$HT_{2A}$ | • | 0471 |
| | 5-$HT_{2B}$ | • | 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-$HT_3$ | | 0411 |
| $Ca^{2+}$ CHANNELS | $Ca^{2+}$ channel (L, dihydropyridine site) | | 0161 |
| $K^+$ CHANNELS | hERG (membrane preparation) | | 1868 |
| | $K_v$ channel | | |
| $Na^+$ CHANNELS | $Na^+$ channel (site 2) | | |
| **NUCLEAR RECEPTORS** | | | |
| STEROID NUCLEAR RECEPTORS | AR | | |
| | GR | | |
| **KINASES** | | | |
| CTK | Lck kinase | | |
| **OTHER NON-KINASE ENZYMES** | | | |
| AA METABOLISM | $COX_1$ | | |
| | $COX_2$ | | |
| MONOAMINE & NEUROTRANSMITTER | acetylcholinesterase | | |
| | MAO-A | | 0443 |
| PHOSPHODIESTERASES | PDE3A | | 2432 |
| | PDE4D2 | | 2434 |

## SafetyScreen44™ Panel

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# *In Vitro* Pharmacological Profilin

## Bowes et al 2012. Nature Reviews: Drug Discovery 11 909-9

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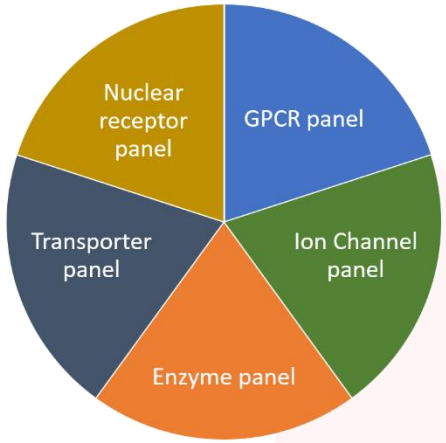
### Mean % inhibition for Bowes 44 panel


### SafetyScreen44™ Panel

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

**No receptor/target-led pharmacological effect**

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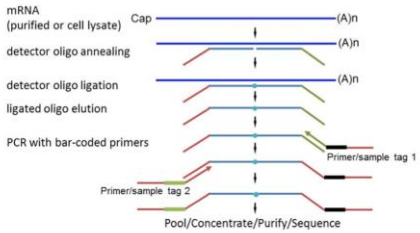
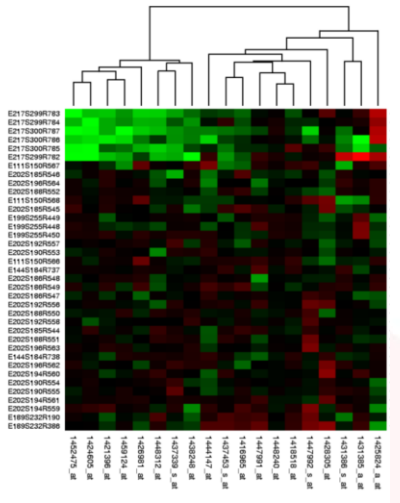
# *In Vitro* Bioactivity: Tempo-Seq Technology

BioSpyder™

1. Defining a safe operating exposure for systemic toxicity using a NOTEL (No Observed 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). Cells treated for 24-hours, BMDExpress2 used to model response.



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

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# *In Vitro* Bioactivity: NOTELs from 3 cell types

- Applying filtering criteria (Farmahin et al., 2017) resulted in fewer than the recommended 20 pathways for NOTEL calculations for each cell line. A very conservative approach of modelling the pathways with the lowest BMDs was used:

| Gene Tests | HepaRG | MCF-7 | HepG2 |
|---|---|---|---|
| $BMD_{10}$ of pathway with the lowest $BMD_{10}$ (µM) | 552.90 | 760.33 | 232.00 |
| $BMDL_{10}$ | 220.92 | 512.84 | 171.25 |
| $BMDU_{10}$ | 911.72 | 1648.51 | 557.20 |

- HepG2 had fewest genes affected and only one pathway showing significant response to treatment (signal transduction)
- In HepaRG cells, cytochrome p450 genes CYP2B6 and CYP2A6 showed the greatest fold changes

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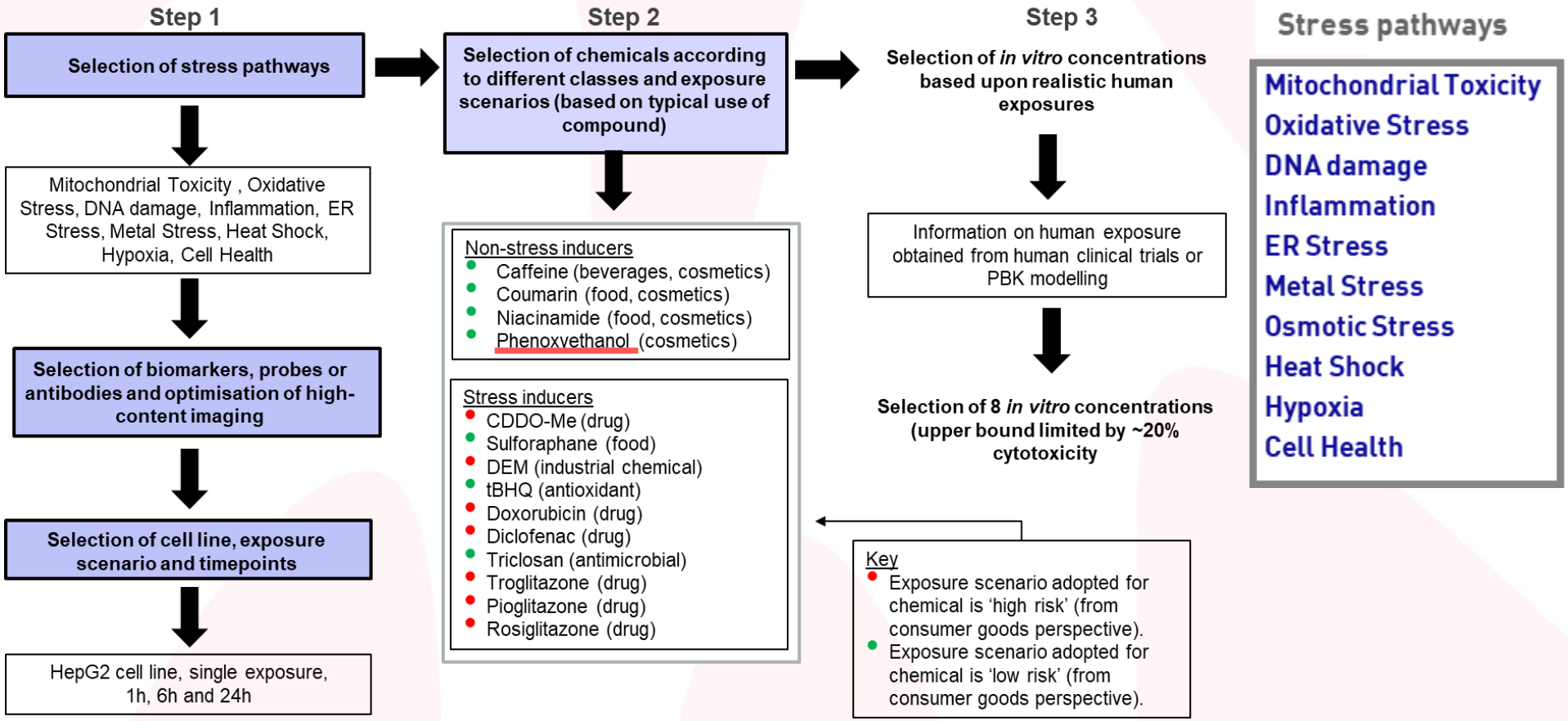
# *In Vitro* Bioactivity: Cell Stress Panel

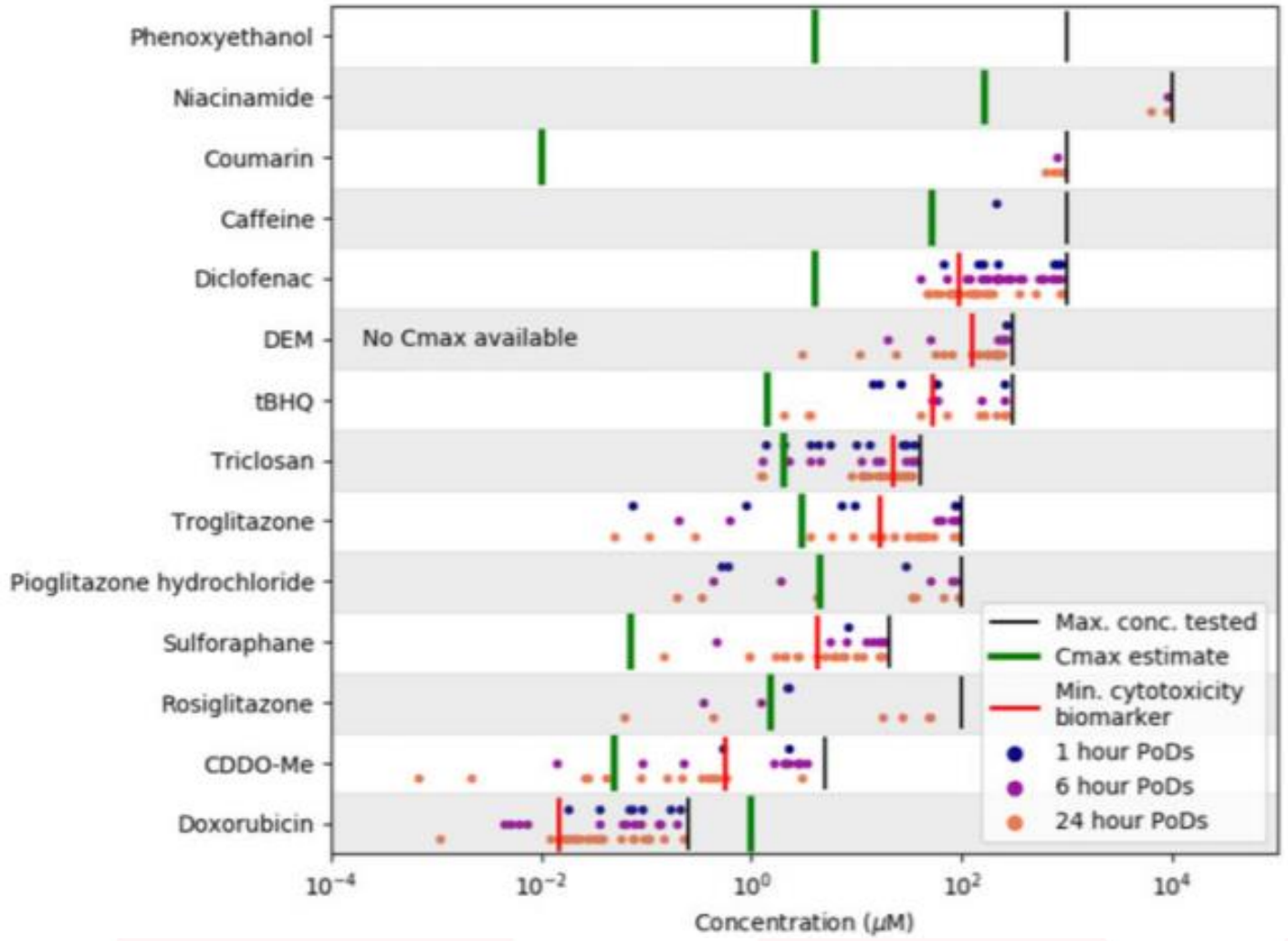
Hatherell et al., 2020 Tox Sci doi: 10.1093/toxsci/kfaa054

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

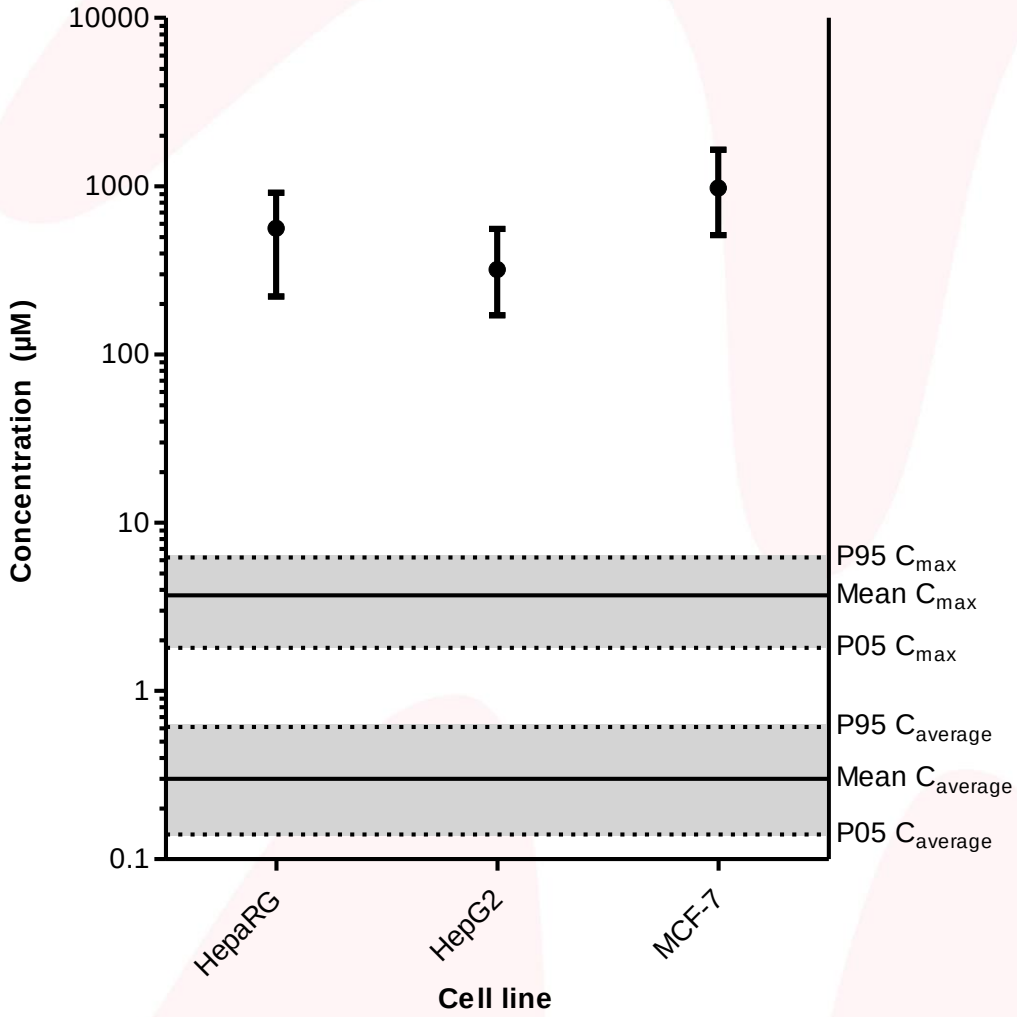


# *In Vitro* Bioactivity: Cell Stress Panel

**Hatherell et al., 2020 Tox Sci doi: 10.1093/toxsci/kfaa054**



# Bioactivity: Exposure comparison for phenoxyethanol




Comparison of 24-hour pathway NOTELs (BMDL10, BMD10 and BMDU10) for phenoxyethanol in 3 cell lines with exposure predicted by population PBK modelling. Dot represents BMD10, error bars show 5th and 95th percentile BMD (BMDL10 and BMDU10 respectively). The lowest pathway BMDL10 (HepG2) was 28 and 280 times higher than the 95th percentile Cmax and Caverage values respectively.

# *In vitro* kinetics refinements

- 24-hour full dose-response performed on phenoxyethanol only
- Was PAA formed in these studies?

**MCF-7, HepG2 and HepaRG cells cultured**

- Dosed with phenoxyethanol at 10, 30, 100, 300 or 1000 µM

**Phenoxyethanol and PAA analysed**

- In cell lysate and medium

**Concentration determined**

- Extracellular
- Cellular

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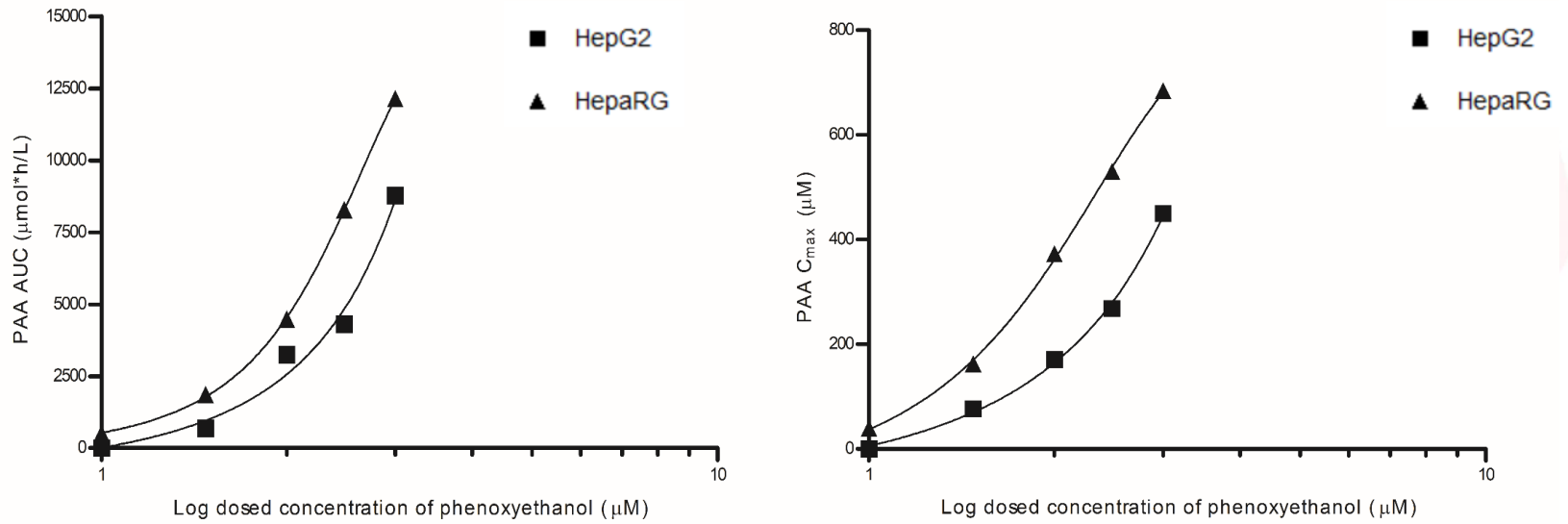
# Formation of PAA in HepG2 and HepaRG cells




**HepG2, HepaRG and MCF-7 cells were incubated at approximately the same seeding density and under the same conditions used in the cell stress and whole genome transcriptomics assays, and dosed with phenoxyethanol at concentrations of 0 (control), 10, 30, 100, 300 or 1000 µM for 0, 1, 3, 6 or 24-h. Cmax and AUC values for cell-associated PAA shown. Negligible formation of PAA in MCF-7 cells.**

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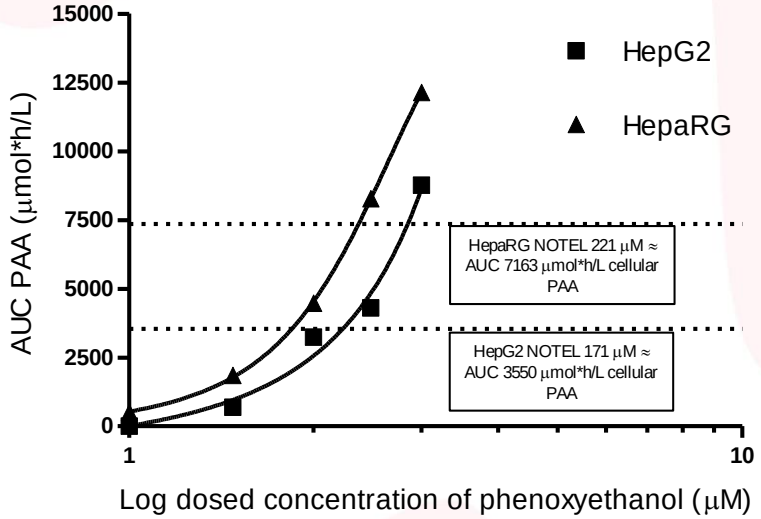
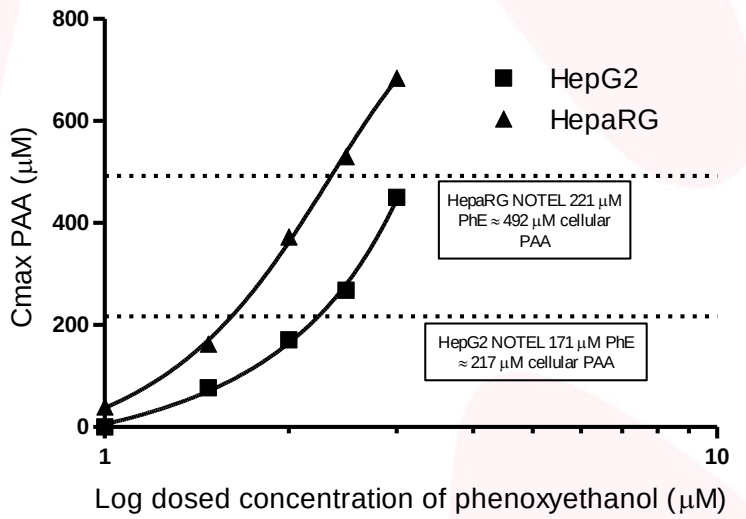
# Handling of major stable metabolite

Phenoxyacetic acid: *In vitro* AUC against log nominal *in vitro* dose




Phenoxyacetic acid: *In vitro* $C_{max}$ against log nominal *in vitro* dose




## Interpolation of PAA Cmax and AUC at NOTEL for phenoxyethanol

# Margin of internal exposures/Bioactivity Exposure Ratios

| Chemical | Scenario | Human Exposure | | PoD | | MoIE/BER | |
|---|---|---|---|---|---|---|---|
| | | $AUC_{24}$ | $C_{max}$ | $AUC_{24}$ | $C_{max}$ | $AUC_{24}$ | $C_{max}$ |
| | | µmol*h/L | µM | µmol*h/L | µM | | |
| PE | Worst case | 15 | 6.2 | 3215 | 171 | 214 | 28 |
| PE | Mean | 7.3 | 3.7 | 4381 | 232 | 600 | 63 |
| PE | Best case | 3.3 | 1.8 | 10708 | 557 | 3245 | 309 |
| PAA | Worst case | 1569 | 69 | 3550 | 217 | 2 | 3 |
| PAA | Mean | 789 | 36 | 4206 | 249 | 5 | 7 |
| PAA | Best case | 312 | 15 | 6573 | 359 | 21 | 24 |

Worst case = BMDL/P95 Exposure; Mean = BMD/Mean Exposure; Best case = BMDU/P5 Exposure

# Key uncertainties

- Range of biomarkers assessed (when do you have enough data?)
- *In vitro* kinetics
- Duration of studies (is 24-h adequate?)
- Point of departure (limited number of cell lines)

How protective is the assessment? (Table 10)

| | Exposure (following use of ingredient at 1% in body lotion) | PoD | MoS/BER |
|---|---|---|---|
| 'Traditional' Risk Assessment* | 1.23 mg/kg/day | 357 mg/kg/day | 290 |
| NGRA based on $C_{max}$ and NOTEL | 6.2 µM | 171 µM | 28 |
| NGRA based on $AUC_{24}$ and NOTEL | 15 µmol*h/L | 3215 µmol*h/L | 214 |

* Based on rabbit dermal 90-day study (SCCS Opinion on Phenoxyethanol)

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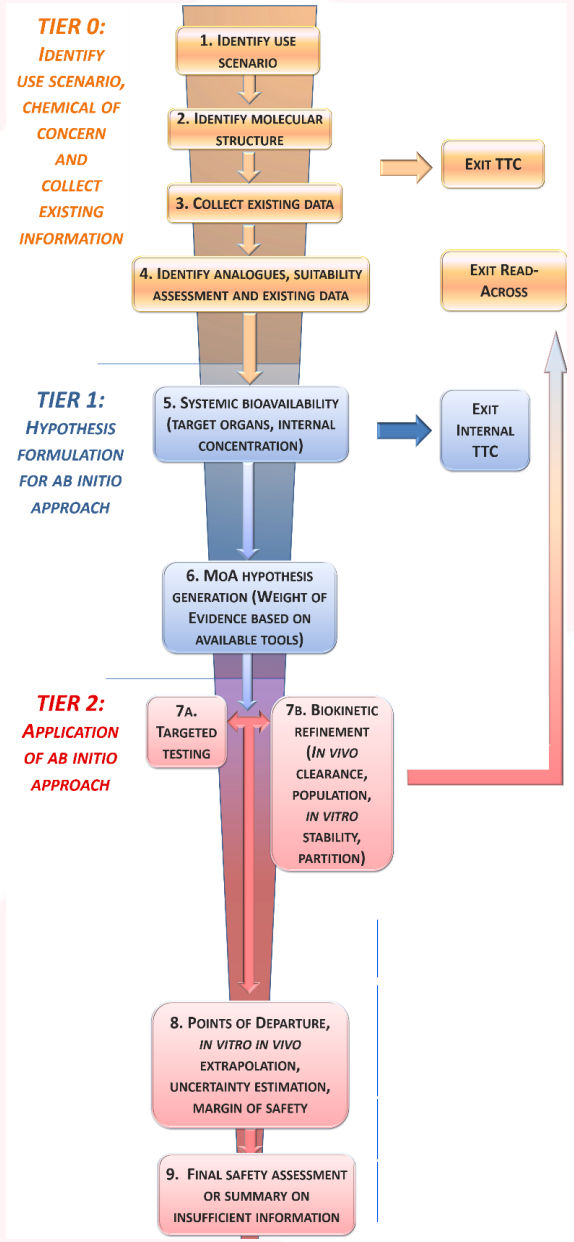
## *Figure 1 How the data used in this case study map to the Next Generation Risk Assessment workflow for systemic effects, and the order in which the case study was performed (Berggren et al., 2017)*




SCCS Notes of Guidance 90th percentile exposure to body lotion, ingredient present at 1%

Existing data harvested from PubChem and ToxCast, no animal data considered in the evaluation

TTC or read across was not a focus for this case study

PBK model developed using literature inputs: no *in vitro* data were generated in Tier 1.

Possible metabolic products predicted *in silico* using Meteor

*In silico* tools used to supplement existing *in vitro* data to try to identify any modes of action of concern:

Targeted testing: High throughput transcriptomics in HepG2, HepaRG and MCF-7 cells; cell stress panel in HepG2 cells; *in vitro* pharmacological profiling.

Biokinetic refinement including population modelling, confirmatory *in vitro* clearance data, confirmatory *in vitro* metabolite characterization in primary hepatocytes and in cells used in targeted testing.

Margins of internal exposure based on $C_{max}$ and $AUC_{24}$ of both phenoxyethanol and the stable acid metabolite

# Final Case Study Conclusions

*"This case study illustrates an ab initio risk assessment of a cosmetic ingredient based on the tools and approaches currently available, and provides a possible approach to evaluating major metabolite. Although the calculated MoIEs were above 1, which indicated that in vitro bioactivity was not seen at consumer-relevant concentrations, there were several uncertainties in the risk assessment which need to be addressed in future work.*

*More case studies on both high and low risk substance exposures using these tools and approaches will further help to put the MoIE values obtained into context, and further embed the application of NGRA to cosmetics."*

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# Extra reading....

## Baltazar *et al* (2020) A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products. Toxicological Sciences, 176, 236-252

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

TOXICOLOGICAL SCIENCES, 176(1), 2020, 236–252

doi: 10.1093/toxsci/kfaa048
Advance Access Publication Date: April 10, 2020
Research article

### A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products

Maria T. Baltazar,[1] Sophie Cable, Paul L. Carmichael, Richard Cubberley, Tom Cull, Mona Delagrange, Matthew P. Dent, Sarah Hatherell, Jade Houghton, Predrag Kukic, Hequn Li, Mi-Young Lee, Sophie Malcomber, Alistair M. Middleton, Thomas E. Moxon, Alexis V. Nathanail, Beate Nicol, Ruth Pendlington, Georgia Reynolds, Joe Reynolds, Andrew White, and Carl Westmoreland

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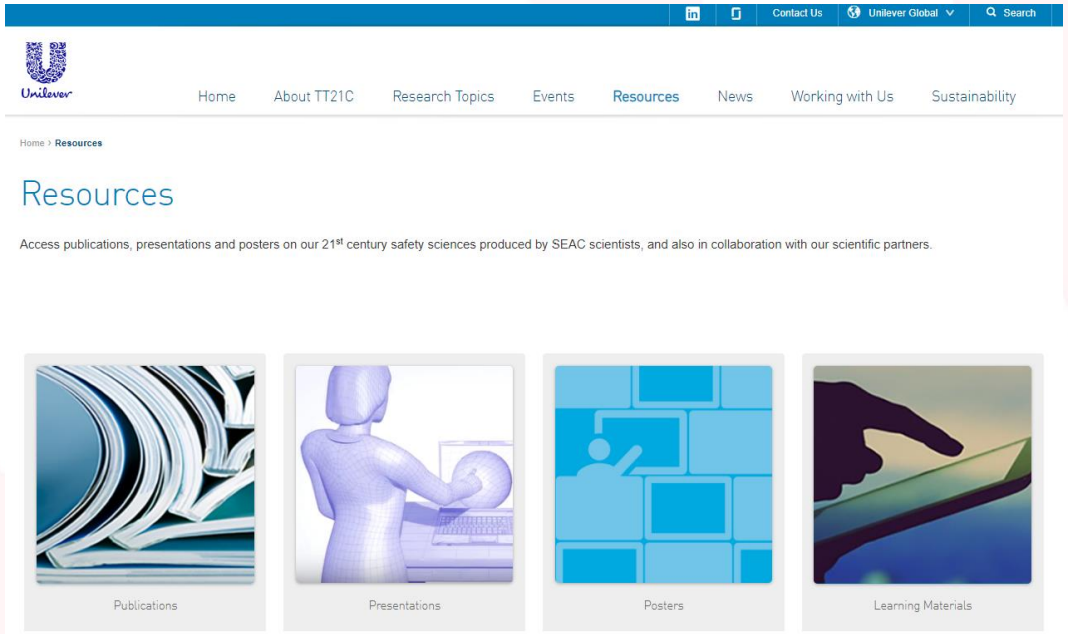
**ABSTRACT**

Next-Generation Risk Assessment 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. These principles were applied to a hypothetical safety assessment of 0.1% coumarin in face cream and body lotion. For the purpose of evaluating the use of NAMs, existing animal and human data on coumarin were excluded. Internal concentrations (plasma $C_{max}$) were estimated using a physiologically based kinetic model for dermally applied coumarin. Systemic toxicity was assessed using a battery of *in vitro* NAMs to identify points of departure (PoDs) for a variety of biological effects such as receptor-mediated and immunomodulatory effects (Eurofins SafetyScreen44 and BioMap Diversity 8 Panel, respectively), and general bioactivity (ToxCast data, an *in vitro* cell stress panel and high-throughput transcriptomics). In addition, *in silico* alerts for genotoxicity were followed up with the ToxTracker tool. The PoDs from the *in vitro* assays were plotted against the calculated *in vivo* exposure to calculate a margin of safety with associated uncertainty. The predicted $C_{max}$ values for face cream and body lotion were lower than all PoDs with margin of safety higher than 100. Furthermore, coumarin was not genotoxic, did not bind to any of the 44 receptors tested and did not show any immunomodulatory effects at consumer-relevant exposures. In conclusion, this case study demonstrated the value of integrating exposure science, computational

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# Thank you!

## LRSS website:

https://www.lrsscosmeticseurope.eu/

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# THANK YOU!


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