NON-ANIMAL APPROACHES FOR COSMETIC SAFETY ASSESSMENT

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COSMETIC SAFETY - INDIA

- Animal testing ban in cosmetics across the world
- BIS updating the Methods of Test for Safety Evaluation of Cosmetics (IS: 4011)

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www.bis.org.in www.standardsbis.in

IS 4011 : 2018

Indian Standard

METHODS OF TEST FOR
SAFETY EVALUATION OF COSMETICS

ANNEX B

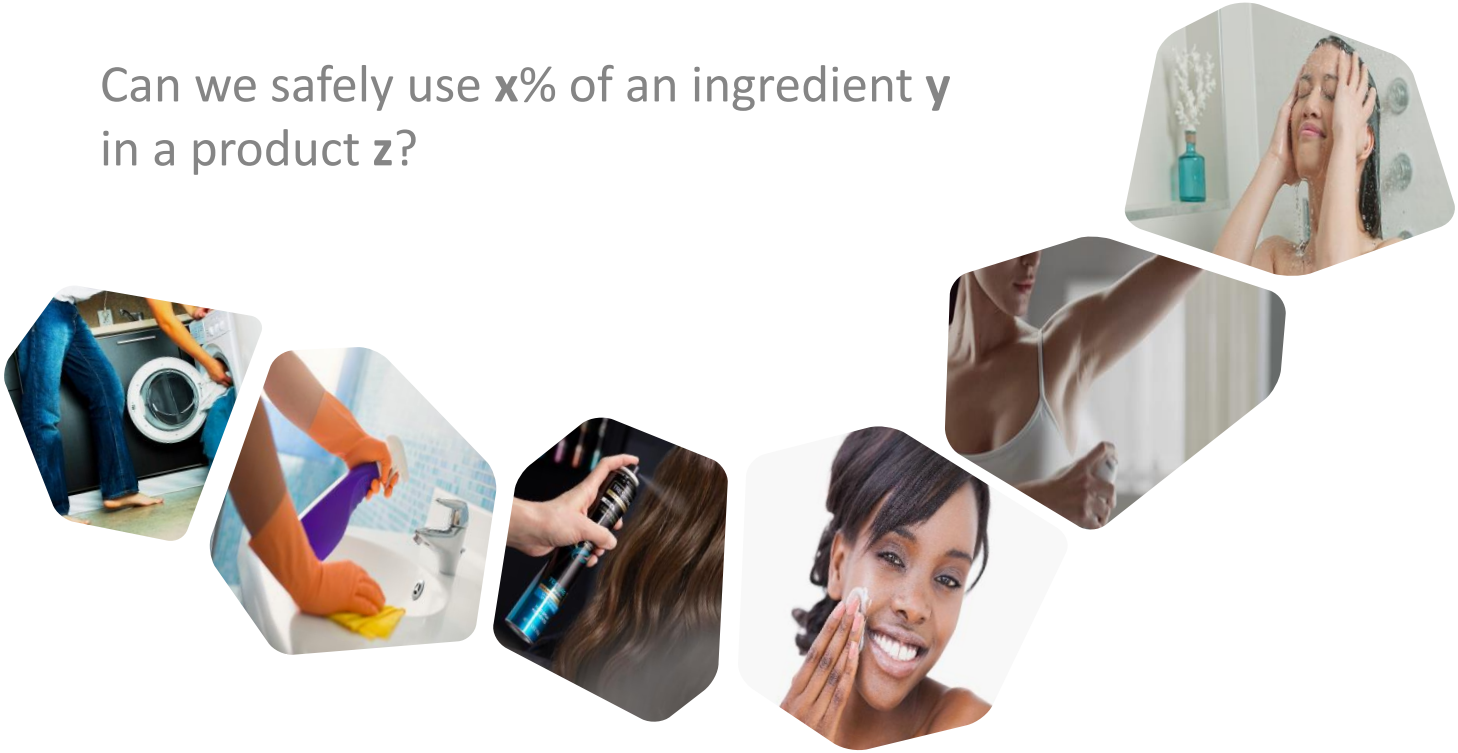
[*Table* 1, *Sl No.* (iii)]

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)

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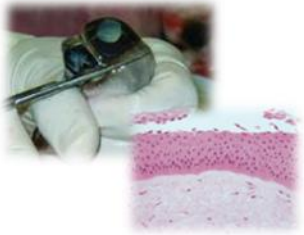
https://ec.europa.eu/health/sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_224.pdf

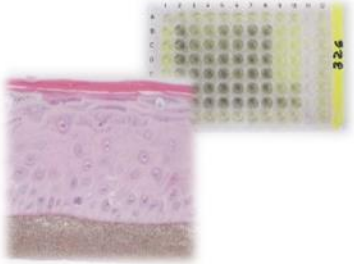
OECD TESTS THAT DON'T USE ANIMALS: USED FOR MANY END-POINTS

OECD TG438



OECD TG437

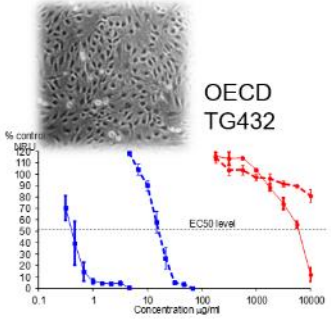
Eye Irritation



OECD TG430/431

OECD TG439

Skin Corrosion/Irritation

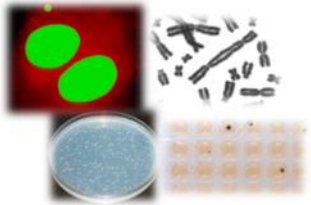


OECD TG432

Phototoxicity

OECD TG487

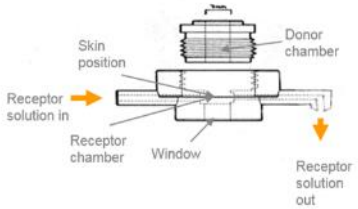
OECD TG473



OECD TG471

OECD TG476

Genotoxicity

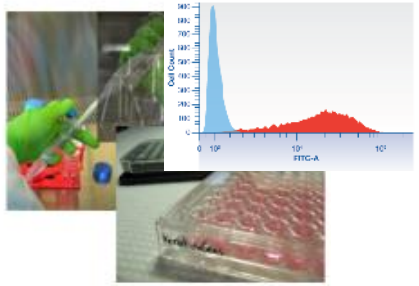


OECD TG428

Skin Penetration

OECD TG442C

OECD TG442E



OECD TG442D

Skin Sensitisation

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

Computational Toxicology 7 (2018) 20–26

Contents lists available at ScienceDirect

Computational Toxicology

journal homepage: www.elsevier.com/locate/comtox

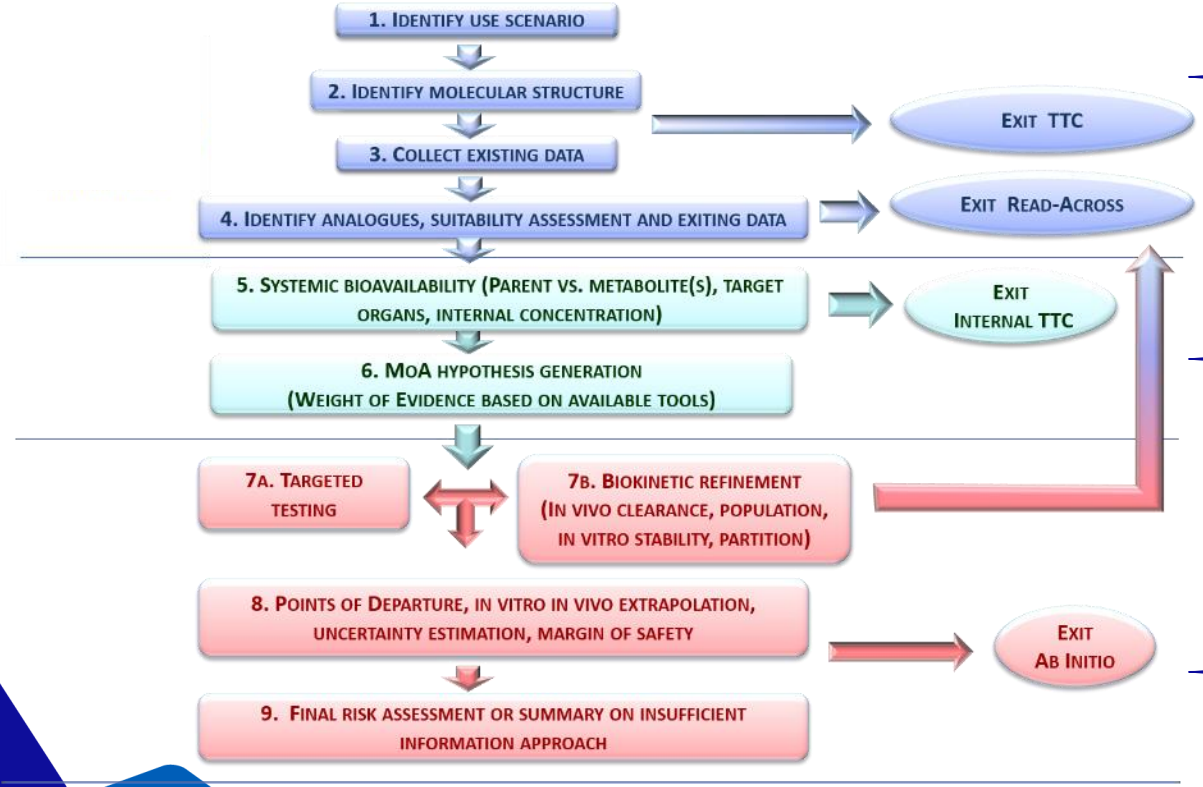
Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients

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[l], Nashira Vieira O'Reilly Cabral Posada[l], Craig Weiss[p], Hajime Kojima[f]

MAXIMISING USE OF EXISTING INFORMATION AND NON-ANIMAL APPROACHES

- All available safety data (of suitable quality, appropriate dates)
  - public domain, historical in-house data, supplier data etc.
  - chemistry data, *in vitro* data, clinical data, epidemiological data, animal toxicology data, etc.
- Exposure-based waiving approaches
- History of safe use
- Read across
- Use of existing OECD *in vitro* approaches
- Next Generation Risk Assessment: Use of NAM (ICCR Principles)

ONE EXAMPLE NGRA WORKFLOW – THE NAMS USED



Read across

Exposure-based waiving

In silico tools

Metabolism and metabolite identification

Physiologically-based kinetic modelling

In chemico assays

'Omics

Reporter gene assays

In vitro pharmacological profiling

3D culture systems

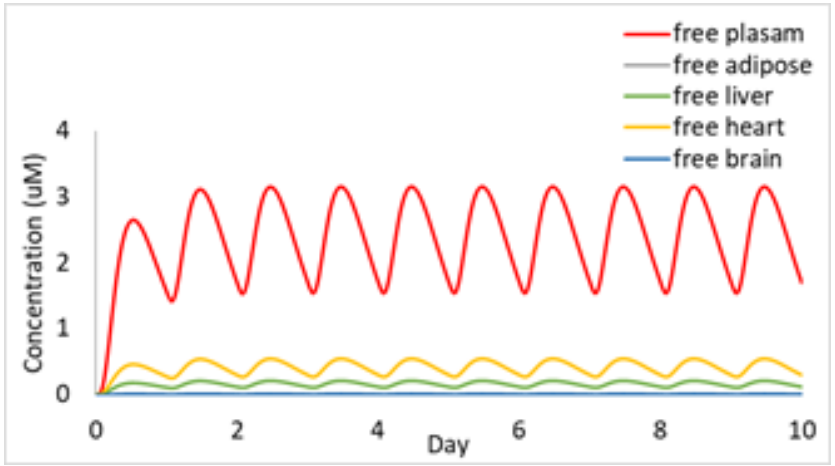
Organ-on-chip

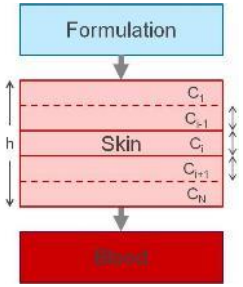
Pathways modelling

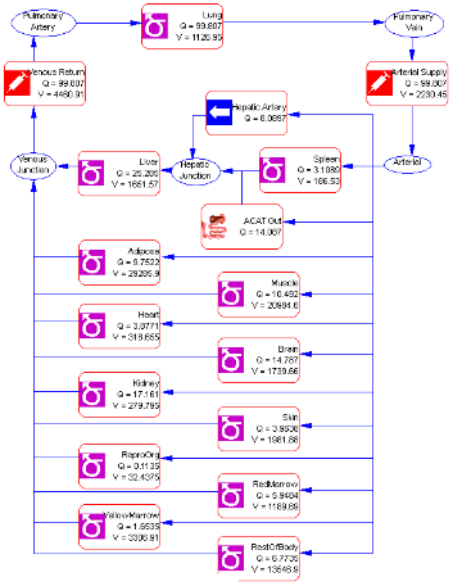
Human studies

Berggren et al., 2017

“THE ASSESSMENT IS EXPOSURE LED” – PBK

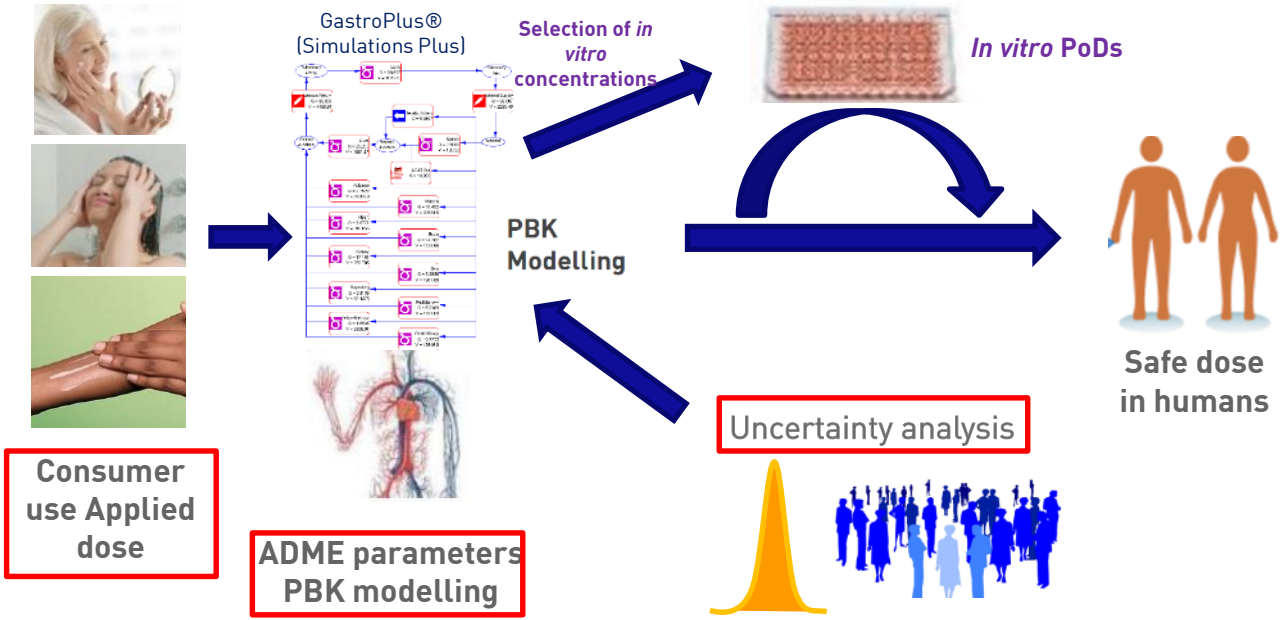




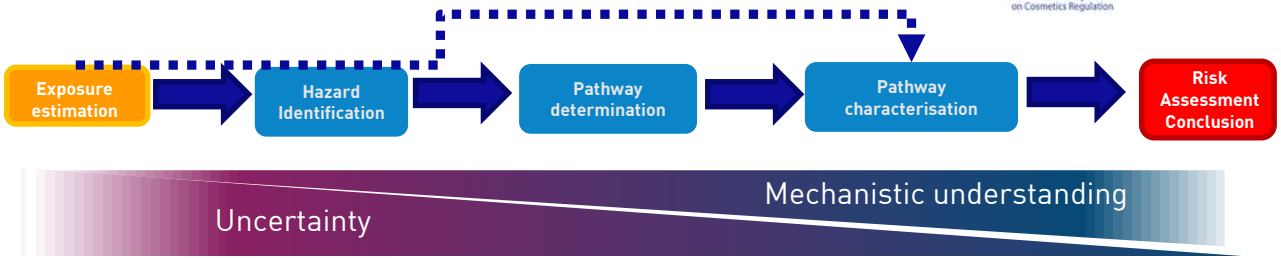


- Predicting systemic exposure
- Enabling us to select and test relevant doses
- Increased role for clinical work to confirm systemic exposure levels

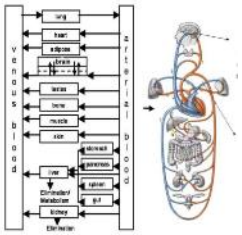
EXPOSURE IN NEXT GENERATION RISK ASSESSMENT



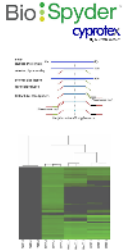
“USING A TIERED AND ITERATIVE APPROACH”

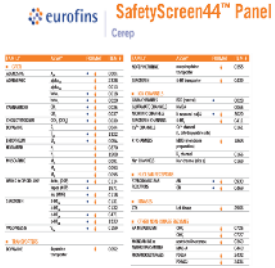


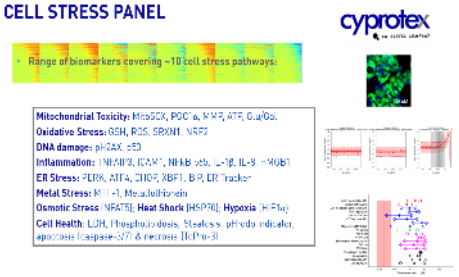
New approach methodologies (NAMs)



PBK models







HYPOTHETICAL CASE STUDY

INCLUSION OF 0.1% COUMARIN IN FACE CREAM TO BE MARKETED IN **EUROPE**

Safety assessment required

FRAMEWORK FOR THE COUMARIN CASE STUDY

Uncertainty

Mechanistic Understanding

Tier 0

Identify use scenario, chemical of concern and collect existing information

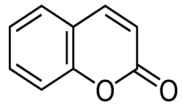
1. Identify Use Scenario

2. Identify Molecular Structure

3. Collect Existing Data

4. Identify analogues, suitability assessment

Existing in vivo and human data excluded. Read across excluded.



Tier 1

Hypothesis formulation for *ab initio* approach

5. Systemic bioavailability (Parent vs Metabolite, target organs, internal concentration).

6. MoA hypothesis generation (WoE based on available tools – *in silico*, *in chemico* and *in vitro*)

Tier 2

Application of *ab initio* approach

7a. Targeted testing

7b. Biokinetic refinement (*in vivo* clearance, population, *in vitro* stability, partition)

8. Point of Departure (PoD), IVIVE, Margin of Safety (MoS), Uncertainty Estimation

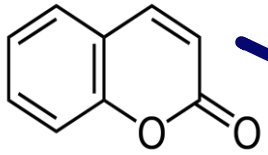
9. Final risk assessment or summary on insufficient information approach

Unilever

TIER 0: IDENTIFY USE SCENARIO AND DETERMINATION OF APPLIED DOSE

1. Identify Use Scenario

Product types	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
Leave on or rinse off	leave on
Exposure duration per occasion	12 hours
For rinse off product, retention factor of finished product on skin [b]	n.a.
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



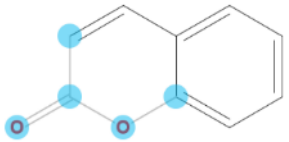
Cramer class III

Exposure to face cream is above TTC (2.3 µg/kg)

Risk assessment progresses to NGRA

TIER 0: IDENTIFY/CHARACTERISE MOLECULAR STRUCTURE

2. Identify Molecular Structure



In silico predictions

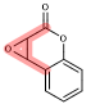
OECD QSAR TOOLBOX

ToxTree

Toxtree

- **Cramer class: High (III)**
- **Protein binding profiler:**
  - Alert for Michael acceptor
  - Alert for acyl transfer
- **DNA binding profiler:**
  - Alert for Michael acceptor

OECD Toolbox: identified alert for SN2 mechanism after oxidation to epoxide



Atlas of MIEs*

- Alert for Cyclooxygenases (Alert COX 2 - Cinnamaldehyde-like) was identified

	OECD Toolbox					ToxTree				DEREK NEXUS					MIE Atlas*					
	Mutagenicity	Endocrine Activity	Chromosome Damage	Protein Binding	DNA Binding	Mutagenicity	Genotoxicity	Protein Binding	DNA Binding	Mutagenicity	Genotoxicity	Endocrine Activity	Chromosome Damage	Skin/Eye irritation	MAO A 2a	MAO A 2b	COX 2	MAR (1,2,3)	PDE 3A 4	A1a ADR
Coumarin	Y	-	-	Y	Y	-	Y	Y	Y	-	-	-	-	-	-	-	Y	-	-	-
Primary Metabolites *(n=7)*	3	5	1	7	7	4	2	7	7	2	1	1	2	1	1	2	5	1	-	-
Secondary Metabolites *(n=13)*	-	7	-	13	10	-	-	13	13	-	2	1	5	3	-	6	6	7	2	1
Tertiary Metabolites *(n=3)*	-	3	3	3	2	-	-	3	3	1	-	-	-	-	-	2	-	-	-	-

*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

Note: predictions that were based on coumarin's animal data were excluded – predictions from DEREK and TIMES-SS were not taken into account.

TIER 0: COLLECTION OF EXISTING DATA

3. Collect Existing Data

- ToxCast data excluded (considered a 'new' chemical)
- A review of the literature was done to validate predicted metabolites
- Literature review was also used for genotoxicity (overall negative decision)

TIER 1: SYSTEMIC BIOAVAILABILITY OF COUMARIN USING PBK MODELLING

5. Systemic bioavailability (**Parent** vs Metabolite, target organs, internal concentration).

Key output parameters from uncertainty analysis:

Parameter	Face cream (applied 2x/day)
Plasma Cmax total (µM)	0.0023
95th percentile Cmax (µM)	0.0043

0.1% Face cream in Europe, from 70kg male

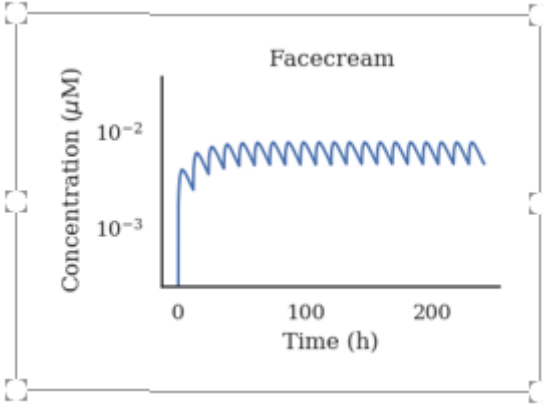
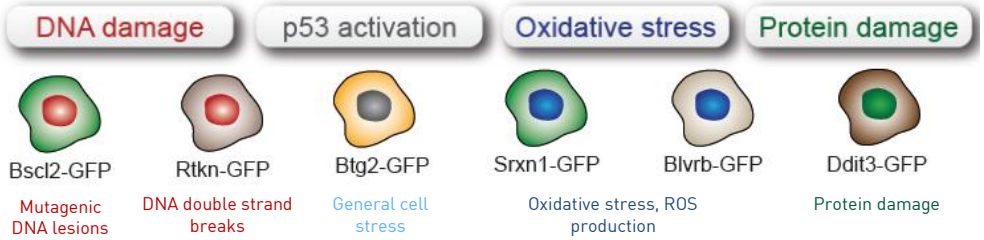


Figure. Physiologically-based kinetic modelling using GastroPlus® v9.5. Parameters were estimated mainly based on experimental data (Clint, fup, bpr, solubility, LogP). Skin penetration parameters were fitted against skin pen data.

GENOTOXICITY SCREENING TOOL

WHY ToxTracker® ?

- Performance of the assay currently exceeds that of the regulatory 2-test battery (Hendriks et al., 2011, 2016)
- Potential to provide Mechanistic Information e.g. Oxidative stress MoA.
- Potential to integrate as part of a battery of NGRA approaches to strengthen confidence in MoA prediction.

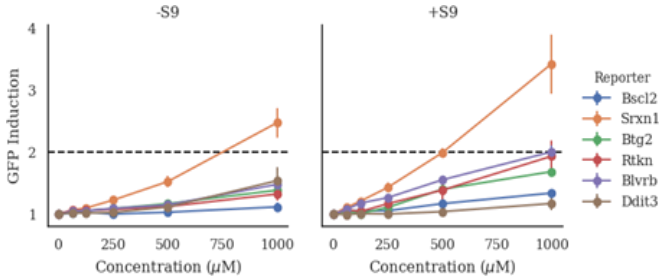


RESULTS FOR COUMARIN

1. ToxTracker assay

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

Positive (>2-fold induction)
Weak activation (1.5 to 2-fold induction)
Negative (<1.5-fold induction)



→ suggestive of reactive coumarin metabolite(s) inducing DNA lesions secondary to oxidative stress, rather than directly interacting with DNA

2. Literature review

The ToxTracker outcome is also supported by in vitro testing data in the literature suggesting that coumarin is not a genotoxic agent of relevance to humans.

- Ames test: Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538, with and without S9. Weak positive results in TA100 +S9, at high concentrations*
- Low clastogenic activity in Chinese hamster ovary cells (Felter, Vassallo, Carlton, & Daston, 2006), at concentrations that exceed current testing guidelines (OECD, 2016)
- No induction of unscheduled DNA synthesis in cultured precision-cut human liver slices (Beamand, Barton, Price, & Lake, 1998)

TIER 1: MOA HYPOTHESIS GENERATION- IN VITRO BIOACTIVITY TOOLS- BIOMAP® DIVERSITY PLUS® 8

Uncertainty

Tier 0

Identify use scenario, chemical of concern and collect existing information

Tier 1

Hypothesis formulation for *ab initio* approach

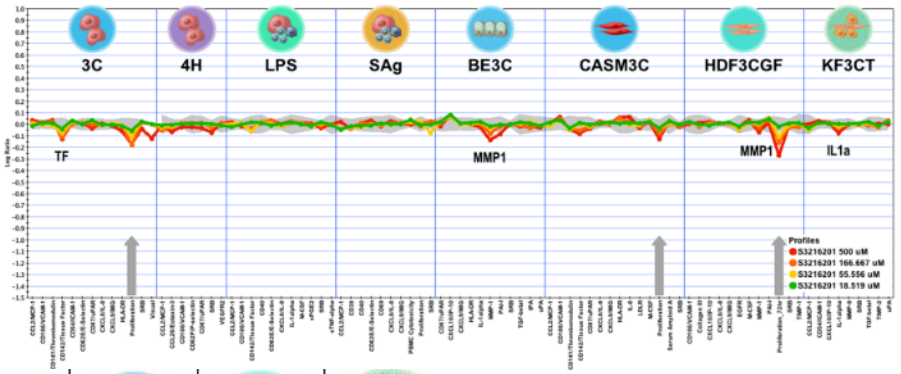
Tier 2

Application of *ab initio* approach

Mechanistic Understanding

6. MoA hypothesis generation (WoE based on available tools – *in silico*, *in chemico* and ***in vitro***)

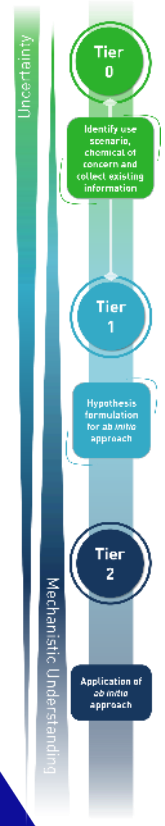
No immunomodulatory effects at relevant concentrations. Data suggest that coumarin is not an anti-inflammatory compound



Cell System	Endothelial (IL1b+TNFa+ IFNy)	Endothelial (IL4+Hist)	PBMC + Endothelial (TLR4)	PBMC + Endothelial (TCR)	Bronchial Epithelial (IL1b+TNFa+ IFNy)	Coronary artery SMCs (IL1b+TNFa+ IFNy)	Fibroblasts (IL1b+TNFa +IFNy +EGF+bFGF +PDGF-BB))	Keratinocytes + Fibroblasts ((IL1b+TNFa +IFNy +TGFb)
LOEL	18.5µM	500µM	>500µM	>500µM	167µM	167µM	56µM	500µM

BioMAP®

TIER 1: MOA HYPOTHESIS GENERATION- IN VITRO BIOACTIVITY TOOLS- CEREP

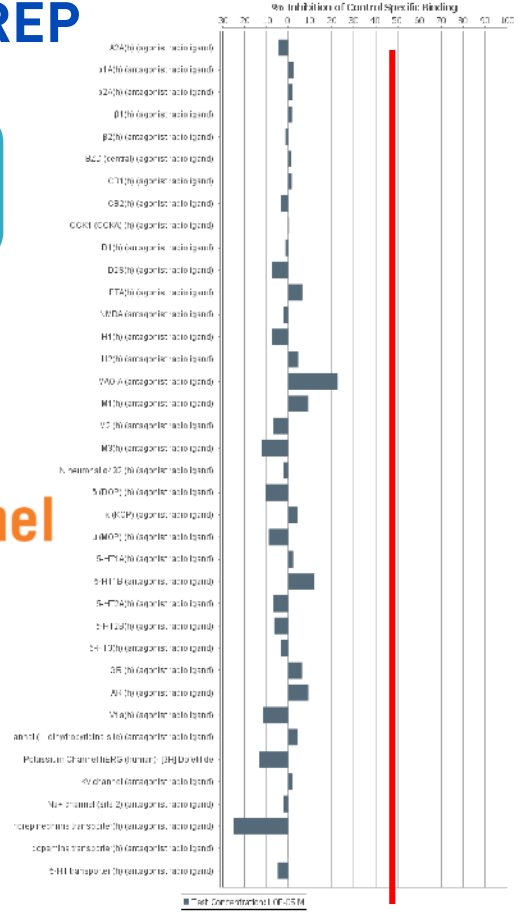


MoA hypothesis generation (WoE based on available tools – *in silico*, *in chemico* and ***in vitro***)

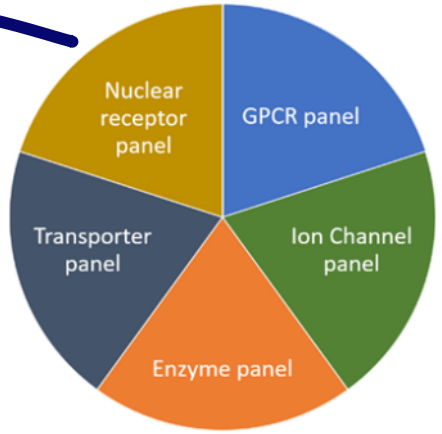
CEREP "Safety Screen 44": (based on Bowes et al 2012).

SafetyScreen44™ Panel

eurofins | Cerep



All binding and enzymatic assay results were negative at 10 uM, including COX-receptor1 and COX-2. No /target-led pharmacological effect



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

TIER 1: MOA HYPOTHESIS GENERATION- IN VITRO BIOACTIVITY TOOLS- CELL STRESS

MoA hypothesis generation (WoE based on available tools – *in silico*, *in chemico* and ***in vitro***)

- **A Bayesian statistical approach** was applied to the dataset to derive a PoD with explicit uncertainty quantification

~40 biomarkers, 3 timepoints, 8 concentrations

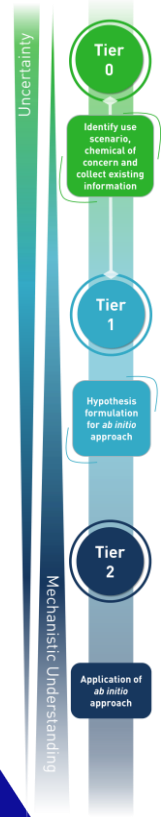
Stress pathways

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

Platform

Technology: High content imaging
Cell line: HepG2
Timepoints: 1, 6 & 24 hours

TIER 1: MOA HYPOTHESIS GENERATION- IN VITRO BIOACTIVITY TOOLS- CELL STRESS



6. MoA hypothesis generation (WoE based on available tools – *in silico*, *in chemico* and ***in vitro***)

Summary table with PoD for cell stress biomarkers:

Biomarker	Cell type	Stress pathway	PoD (µM)	Effect
ATP (6h)	HepG2	Cell health	794	down
ATP (24h)			617	down
Phospholipidosis (24h)	HepG2	Cell health	759	down
GSH (24h)	HepG2	Oxidative stress	851	up
IL-8 (24h)	HepG2	Inflammation	912	down
OCR (1h)	NHEK	Mitochondrial toxicity	62	down
OCR (6h)			468	
OCR (24h)			309	
Reserve capacity (1h)	NHEK	Mitochondrial toxicity	**44**	down
Reserve capacity (6h)			759	
Reserve capacity (24h)			794	

- Only **cellular ATP, GSH, IL-8 and phospholipidosis** showed a dose response in HepG2 cells

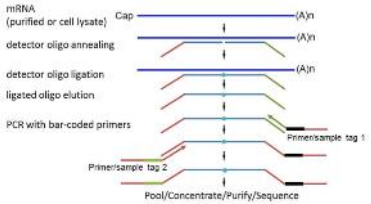
PoD for cell stress biomarkers 24h in HepG2 and NHEK:

BIOSPYDER– TEMPO-SEQ TECHNOLOGY

- High-throughput gene expression profiling
- Performed at BioSpyder with Cyprotex

Defining a safe operating exposure for systemic toxicity using a **NOTEL** (no observed transcriptional effect level)

[Lobenhofer EK, Cui X, Bennett L, Cable PL, Merrick BA, Churchill GA, et al. Exploration of low-dose estrogen effects: identification of No Observed Transcriptional Effect Level (NOTEL). Toxicol Pathol. 2004;32(4):482–92]

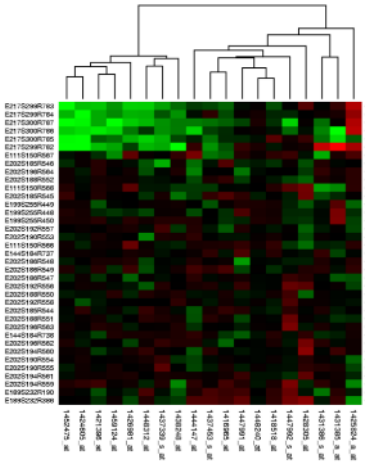


Cell lines (chosen to express a range of relevant receptors)

MCF-7 – human breast adenocarcinoma cell line

HepG2 – human liver carcinoma

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



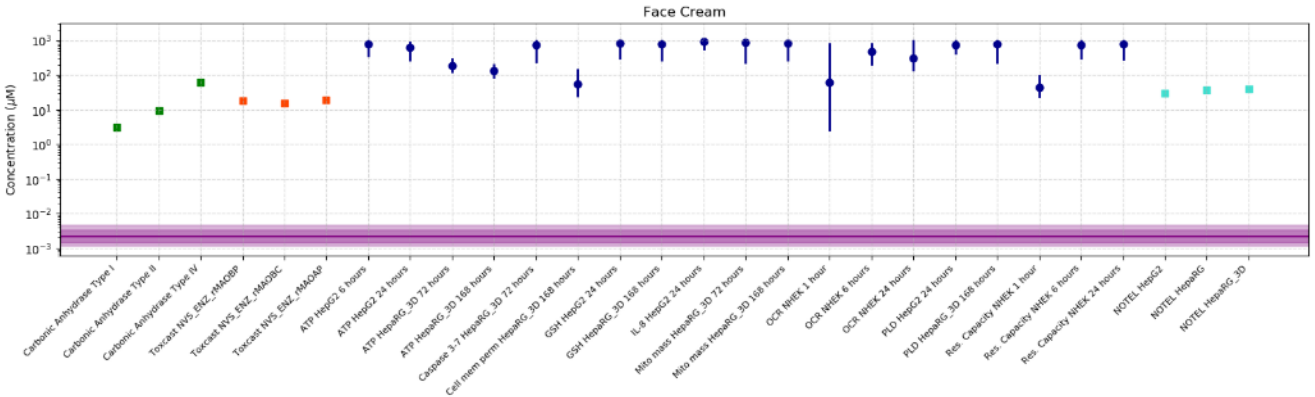
NOTEL* is the derived concentration of a compound that does not elicit a **meaningful** change in gene expression

(i.e. the threshold of the concentration that elicits minimal mechanistic activity).

TIER 2: APPLICATION OF AB INITIO APPROACH – POD AND MOS PLOT

Point of Departure (PoD), IVIVE, Margin of Safety (MoS), Uncertainty Estimation

Face cream 0.1%



Cmax expressed as a distribution:

- Red line= median (50th percentile)

PoDs and plasma Cmax (µM) are expressed as total concentration.

TIER 2: APPLICATION OF AB INITIO APPROACH – RISK ASSESSMENT

Final risk assessment or summary on information generated SO FAR

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

Most conservative Plasma C_{max} 95th percentile

Face cream

MoS considering lowest POD (HTTr HepG2) = 7223

MoS considering highest POD (Cell stress panel NHEK) = 1330

ONGOING WORK TO STRENGTHEN NGRA

- Generation of experimental data to further understand the influence of liver and skin **metabolism** on the risk assessment decisions is ongoing

- **Skin penetration data** is being analysed and incorporated in the PBK model

- Data generated in **metabolically competent cell lines or with metabolic activation** to help reduce uncertainty on potential metabolite-driven effects

- Approaches to analysing and interpreting *in vitro* data and **defining points of departure,** particularly for NOTEL values being further developed

CONCLUSIONS

- ICCR principles help us get to an NGRA decision
  - This case study appears to be protective of human health for a cosmetic product
- Importance of understanding consumer exposure
  - Including the relevance of metabolism
- Constructed from *in silico* modelling approaches and *in vitro* solutions
  - Need to ensure quality/robustness of the non-standard work and to characterise uncertainty to allow informed decision-making

THE HUMANE SOCIETY OF THE UNITED STATES | Our Big Fights | Our Impact | How You Can Help

News

Press Release October 15, 2019

The Humane Society of the United States announces honorees for "To the Rescue!" New York 10th Anniversary Gala

Nov. 15 event to honor Unilever, Patrick McDonnell and the Alex & Elisabeth Lewyt Charitable Trust

NEW YORK—The Humane Society of the United States today announced that consumer goods company Unilever, MUTTS© cartoonist and children's book author Patrick McDonnell and the Alex & Elisabeth Lewyt Charitable Trust will be honored at the 2019 "To the Rescue!" New York 10th Anniversary Gala to benefit and celebrate the organization's animal rescue efforts.

HUMANE SOCIETY INTERNATIONAL

Unilever's commitment to ending animal testing is underpinned by the work of its Safety & Environmental Assurance Center, which has worked since the 1980s to develop and use alternatives to animal tests for assessing safety, e.g. computer-based modelling and cell-based 'in vitro' methods. As part of Unilever's commitment to ending animal testing, they have a growing number of brands that ensure that neither finished products nor the ingredients they use are subject to animal testing by suppliers or by regulatory authorities and are certified as such by animal welfare groups. Unilever was the first of the "top 5" beauty brands to call for a global ban on cosmetic animal testing in partnership with Humane Society International and the HSUS, and is a founding member of the Animal-Free Safety Assessment Collaboration, which works to accelerate global adoption of modern, human-based approaches to safety assessment that will better protect consumers and hasten the replacement of animal testing.

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QUESTIONS?

THANKS TO ALL THE SEAC TEAM AND
OUR MANY EXTERNAL PARTNERS