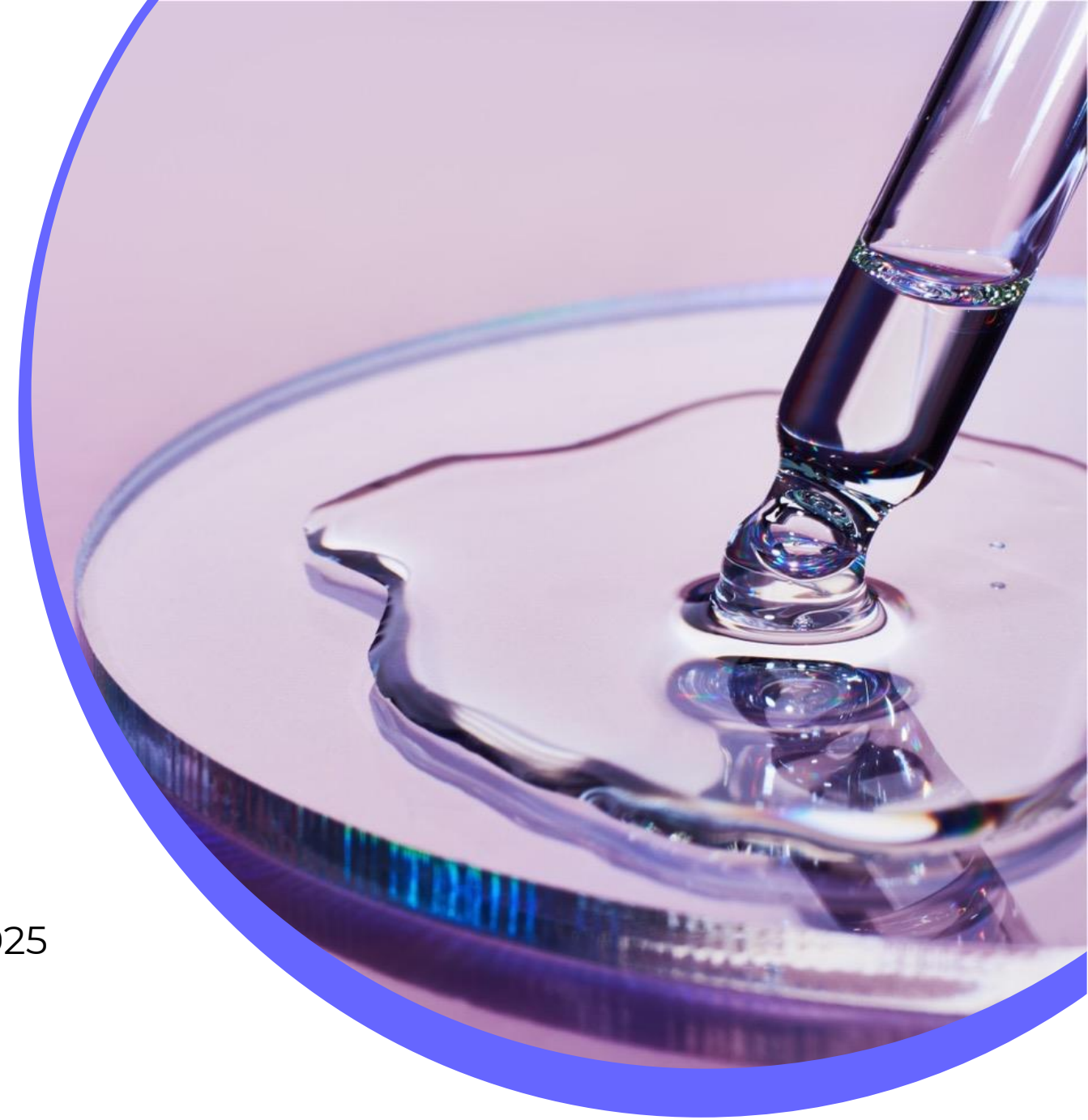
ICCS | INTERNATIONAL COLLABORATION ON COSMETICS SAFETY

Collaborative Efforts to Advance NAMs and NGRA for Cosmetics and their Ingredients

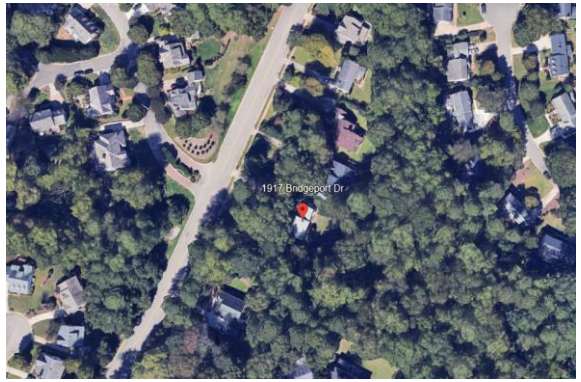


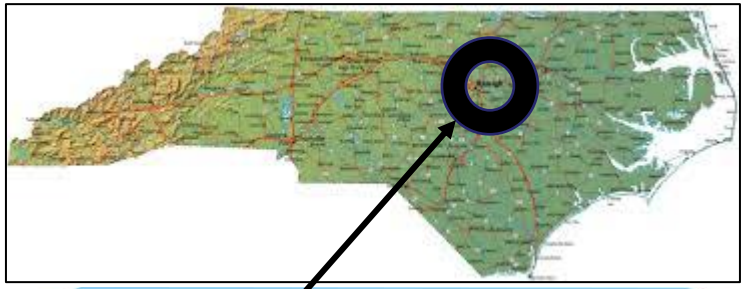
CTFA Safety Assessment Seminar – 28 August 2025

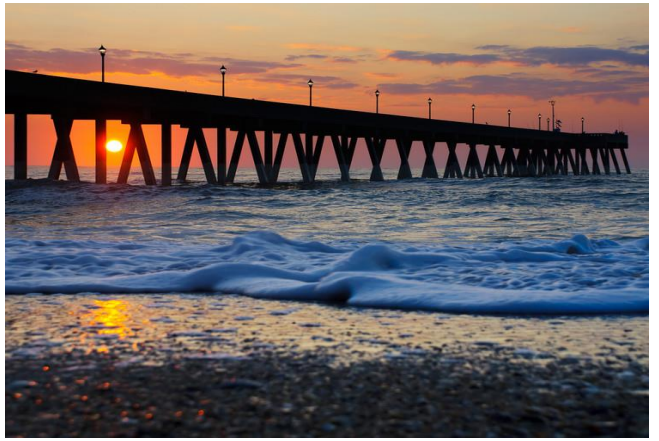
David Allen, PhD, Sr Director, dallen@iccs-cosmetics.org

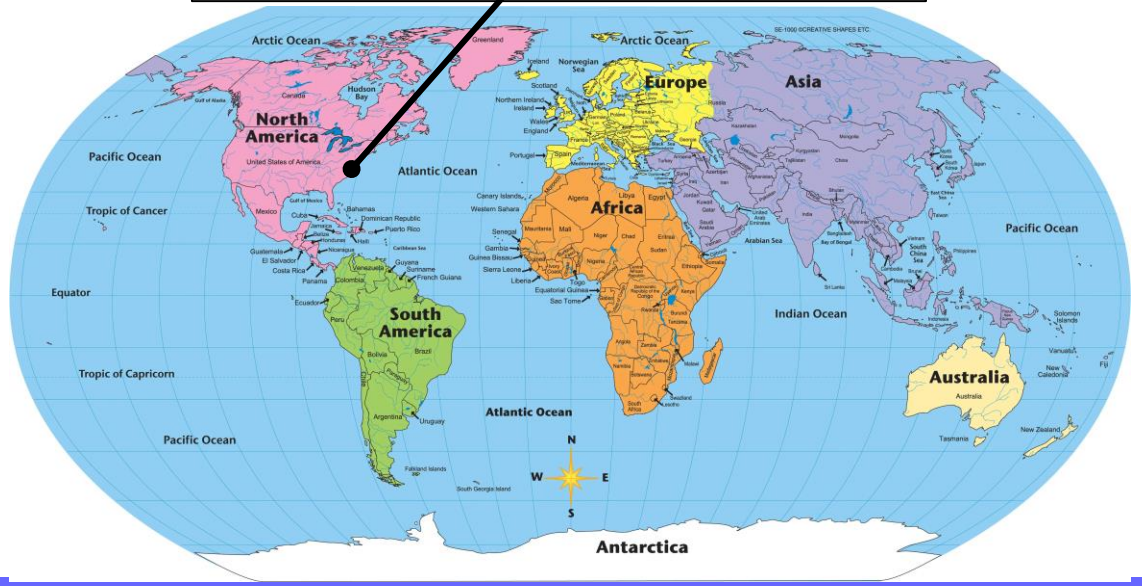
Greetings from Raleigh, North Carolina, USA!



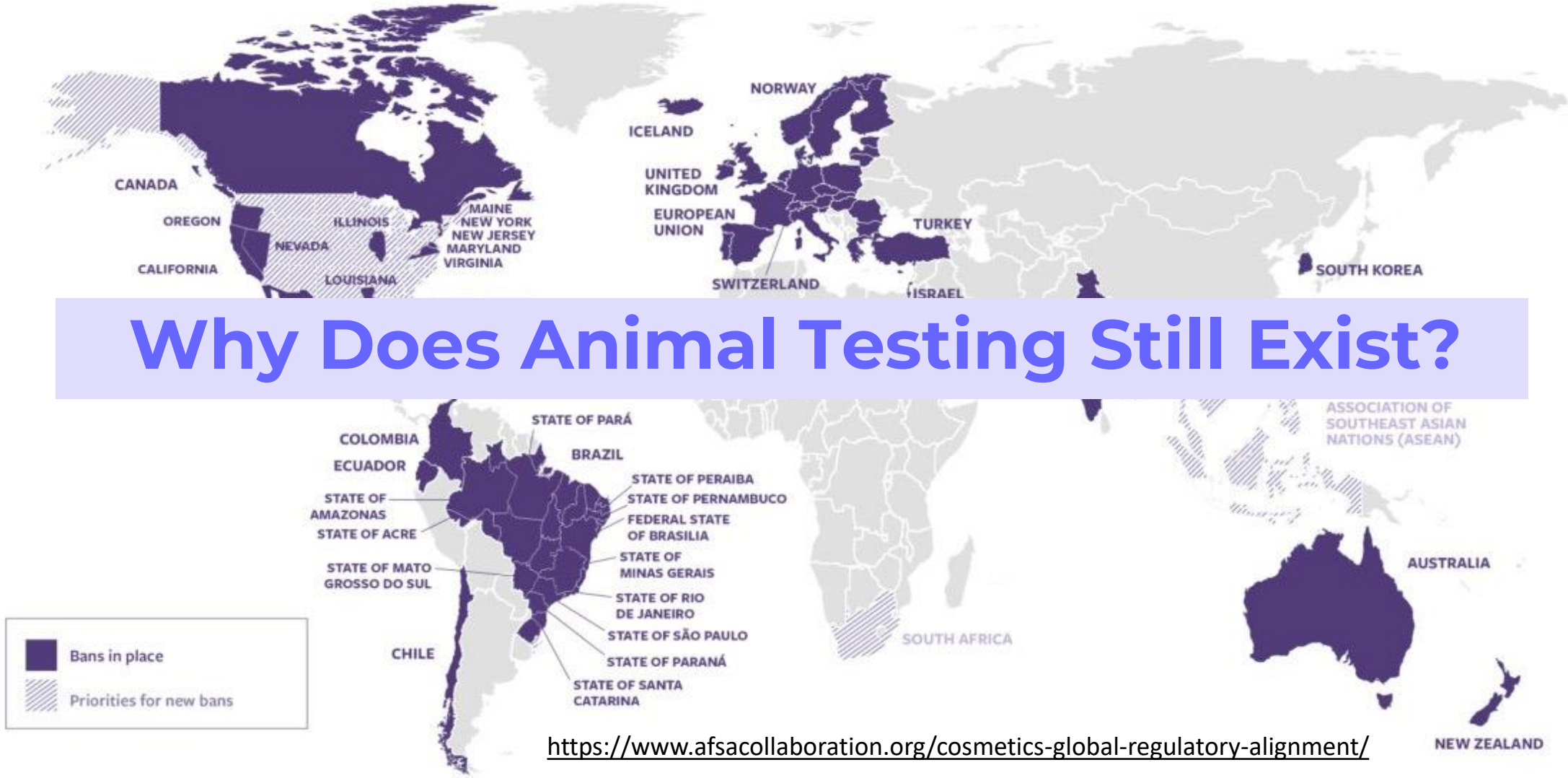








An Increasing Number of Cosmetic Testing Bans Globally



Why Does Animal Testing Still Exist?

https://www.afsacollaboration.org/cosmetics-global-regulatory-alignment/

Cosmetic Regulation Varies Regionally





“Cosmetic” does not have a standardized definition – some are considered drugs in regions





New ingredients, or those with functional properties, require safety assessments

Images: Cosmetics Design

Competing Regulations Within a Region: EU

Chemical Regulation

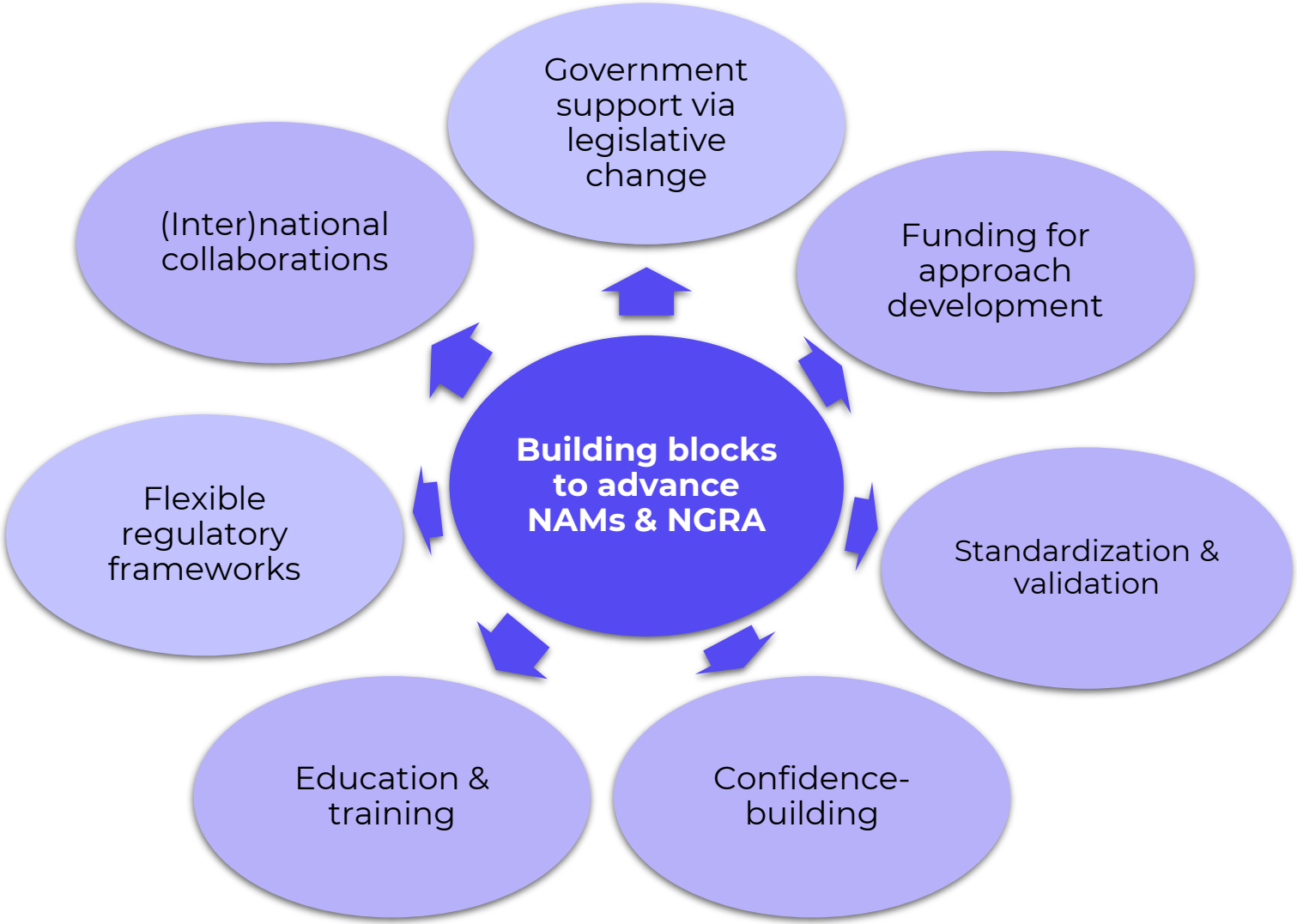
Animal Testing



Cosmetic Regulation

Animal Testing Ban

Critical Components For Change



International Collaboration on Cosmetics Safety (ICCS)



- **Global non-for-profit science organization** headquartered in US
- **Over 40 member organizations** from NGOs, cosmetic & chemic trade and research associations, cosmetic product and ingredient manufacturers
- Dedicated to **advancing animal-free safety assessments** for cosmetic products and **ingredients** through **Regulatory** Engagement, **Education**, and **Science**
- Covering **human health** and **environmental safety**



OUR MEMBERS

The members of our organization come from three sectors: NGOs, Cosmetic & Chemical Trade and Research Associations, and Cosmetic Product and Ingredient Manufacturers.

NGOs

Cruelty Free INTERNATIONAL

HUMANE SOCIETY INTERNATIONAL

IIVS Institute for In Vitro Sciences — Advancing Science & Animal Welfare Together

PETA SCIENCE CONSORTIUM INTERNATIONAL e.V.

Physicians Committee for Responsible Medicine

ICCS

Cosmetic & Chemical Trade and Research Associations

ctpa

ifra THE INTERNATIONAL FRAGRANCE ASSOCIATION

COSMETICS ALLIANCE CANADA

JCIA Japan Cosmetic Industry Association

Cosmetics Europe the personal care association

CASIC Beauty and Cleaning, for Latin-American well-being

EFfCI THE EUROPEAN FEDERATION FOR COSMETIC INGREDIENTS

Personal Care Products Council Committed to Safety, Quality & Innovation

FRAGRANCE CREATORS ASSOCIATION

RIFM RESEARCH INSTITUTE FOR FRAGRANCE MATERIALS

Cosmetic Product and Ingredient Manufacturers

AMOREPACIFIC

EVONIK Leading Beyond Chemistry

LVMH

BASF We create chemistry

HALEON

ORIFLAME SWEDEN

Beiersdorf

Henkel

P&G

innospec

reckitt

COLGATE-PALMOLIVE

Inolex

SHISEIDO

COTY SINCE 1904

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SYENSQO

CRODA

kao Kirei–Making Life Beautiful

TAKASAGO

Edgewell PERSONAL CARE

kenvue

Unilever

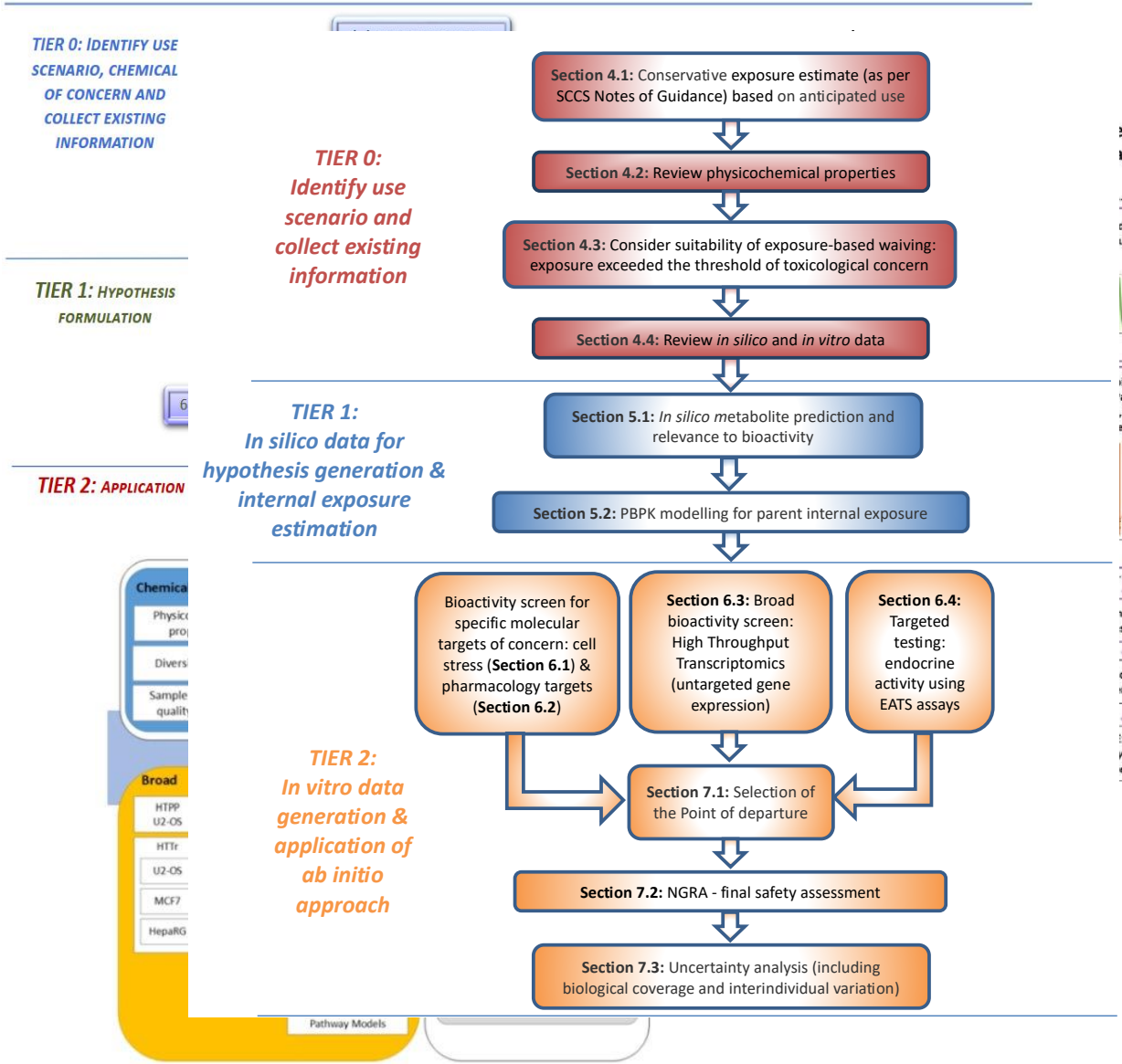
ESTÉE LAUDER COMPANIES

L'ORÉAL

WELLA COMPANY

Frameworks for Next Generation Risk Assessment (NGRA)

- Based on the strategic use of nonanimal methods
- Use scenarios and exposure considered
- Aim is **protection** of human health and the environment, not prediction of animal models
- Acceptance of this requires the demonstration of its consumer-protective application in the form of hypothetical **case studies** using NGRA principles



Cable et al., 2025

9 NGRA Principles from the International Cooperation on Cosmetics Regulation (ICCR)

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


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

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

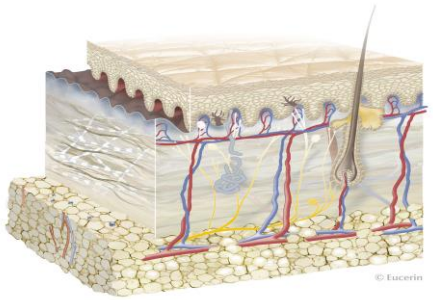
Principles for documenting NGRA:

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

ICCS

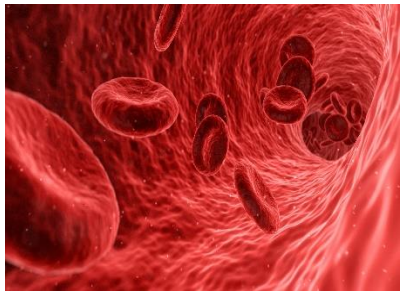
Publication freely available

Tools to characterize human health exposure



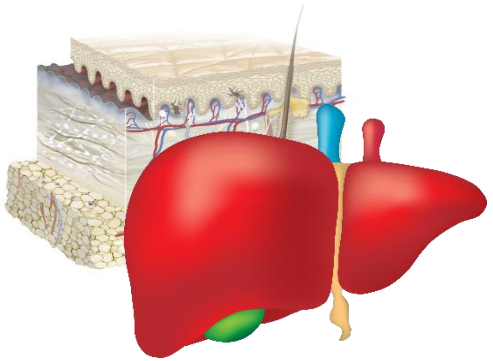
Absorption

- Cell-based assays
- Skin penetration



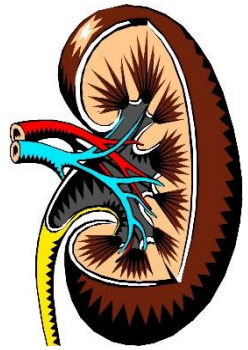
Distribution

- Stability in plasma
- Plasma protein binding
- Blood:plasma ratio



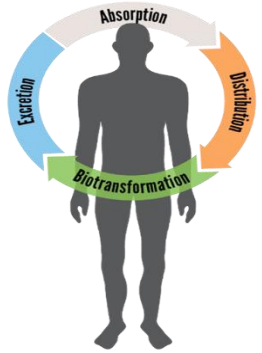
Metabolism

- Skin and liver S9
- Primary keratinocyte assays
- Hepatocyte assays



Excretion

- Renal clearance
- Cells lines containing renal transporters



Pharmacokinetics

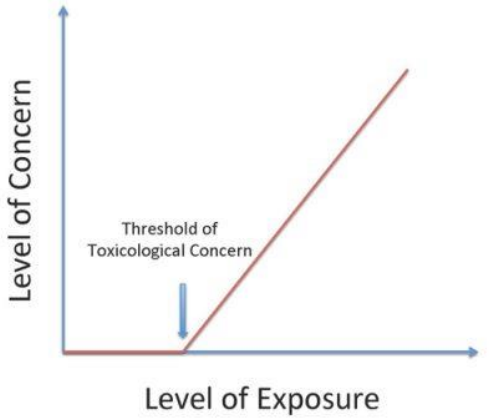
- IVIVE
- PBPK modeling

Exposure Based Waiving: The Threshold of Toxicological Concern (TTC)

1. Chemical Classification

Chemicals are assigned to 1 of 3 “Cramer classes” based on their structural features and predicted metabolism.

- **Class I:** Simple structures, efficiently metabolized, considered low toxicity.
- **Class II:** More complex structures, less innocuous than Class I, but no strong indicators of toxicity.
- **Class III:** Possess structural features that suggest significant toxicity or reactivity.



2. TTC Value Assignment

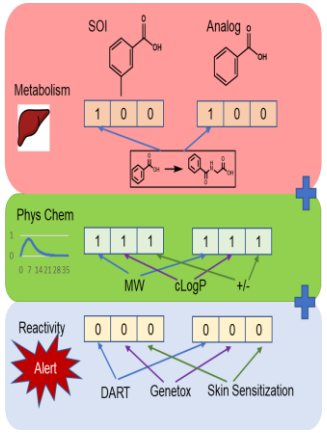
Based on these classifications, a TTC value (a maximum safe daily intake in µg/person/day) is assigned to the chemical.

3. Risk Assessment

If the estimated **human exposure < assigned TTC value**, it is generally considered safe, **avoiding the need for further testing**.

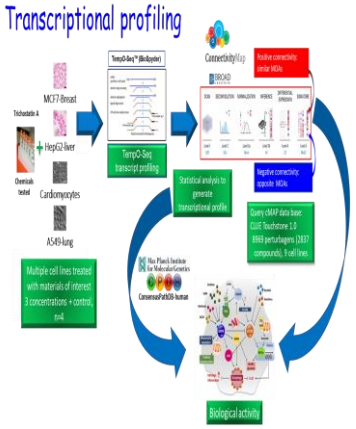
https://doi.org/10.1016/j.yrtph.2021.104964
https://doi.org/10.2903/j.efsa.2019.5708
https://lri.americanchemistry.com/
https://toxedfoundation.org/threshold-of-toxicological-concern

Tools to characterize human health effects



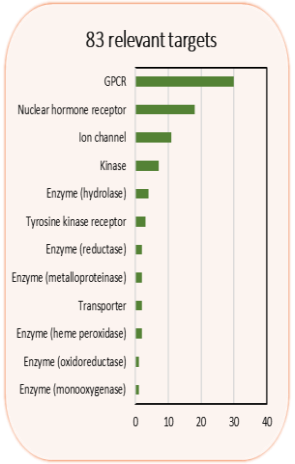
Cheminformatics

- Physicochemical properties
- Read Across



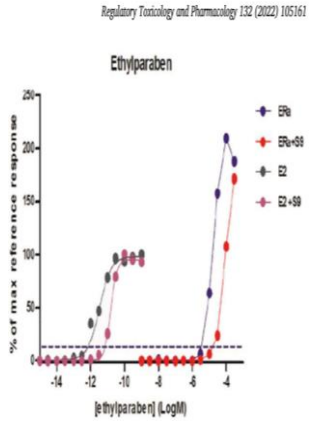
Toxicogenomics

- Transcriptomics
- Various cell types
- Insights into mechanism of action
- Pathway analyses



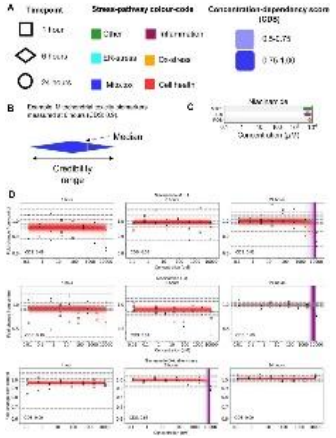
Pharmacology profiling

- Ligand affinity assays
- Targets used in pharma plus cellular functions have been linked to systemic toxicities
- Broad biological coverage



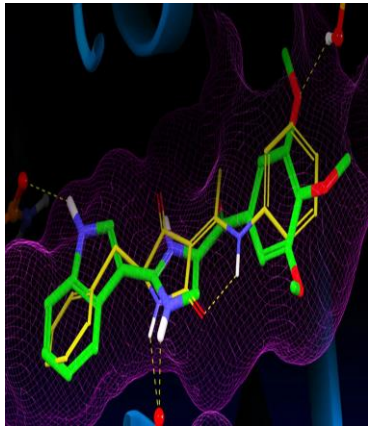
Functional Assays

- Hormone receptor activity
- EATS



Cell Stress Assays

- Pathways related to cell stress and cell health

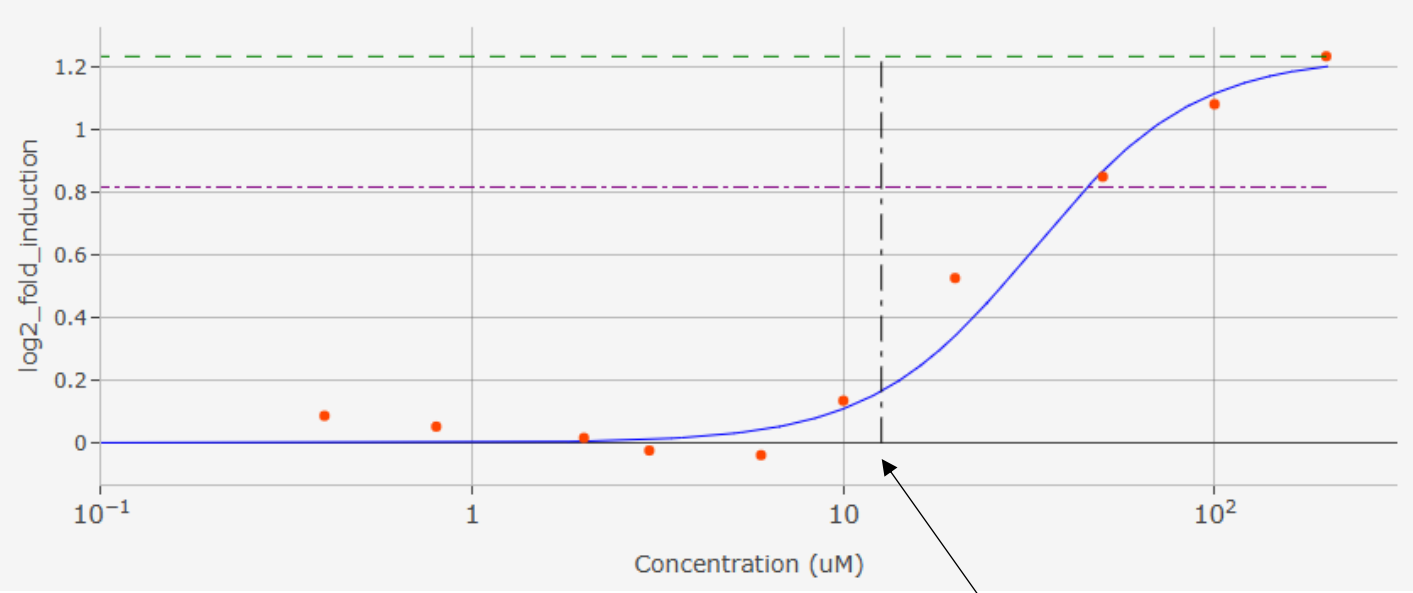


In silico Assays

- QSAR modeling
- Receptor binding

ICCS

Using In Vitro Bioactivity and Exposure Predictions Together



https://ice.ntp.niehs.nih.gov/

Point of Departure (PoD): In vitro concentration at which a biologically relevant response is observed

$$\text{BER} = \frac{PoD}{Estimated\ Human\ Exposure}$$

A LARGE BER

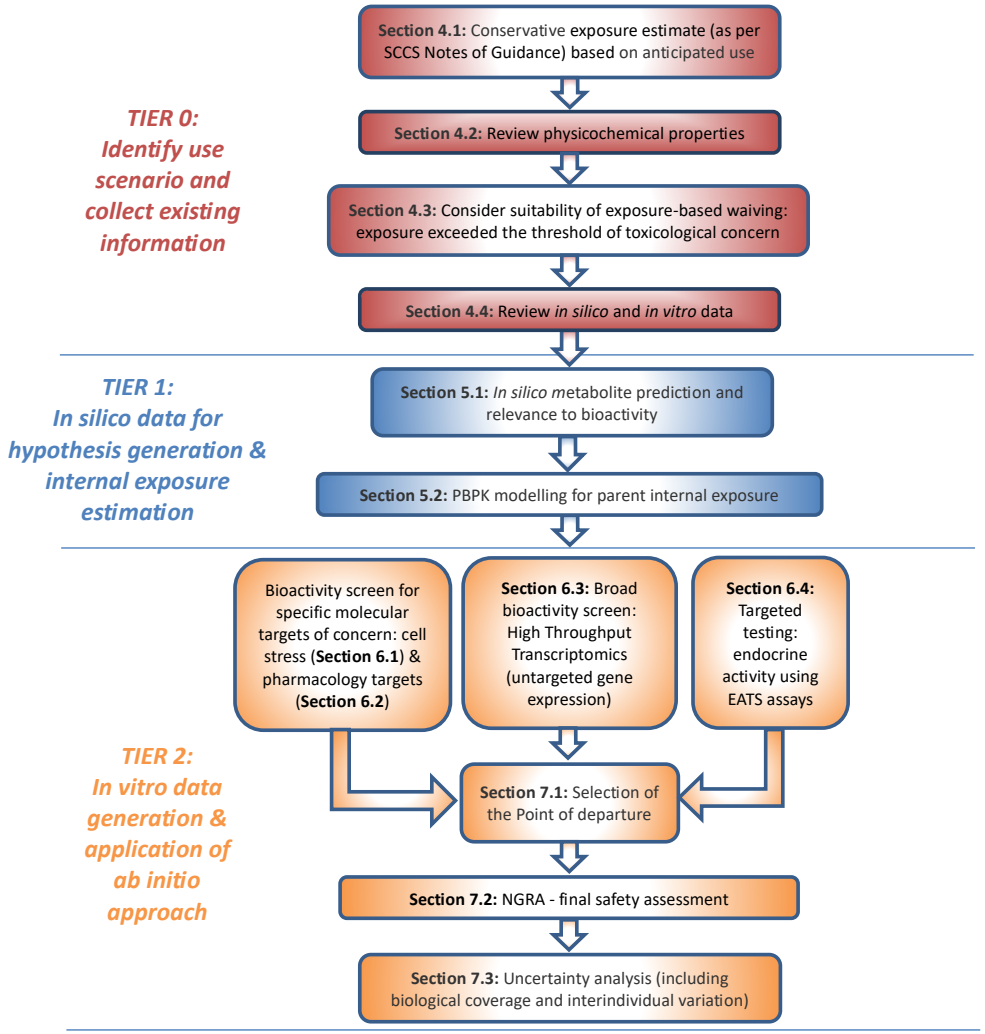
suggests a wide margin of safety

A small BER suggests bioactivity is near estimated exposure levels and further investigation warranted

CASE STUDIES



NGRA Case Study – 1

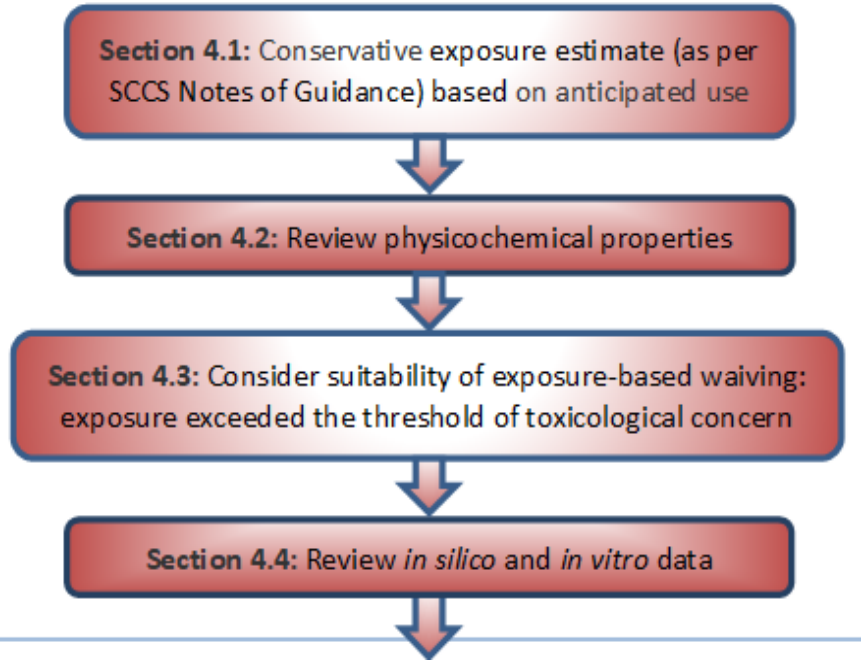




Ab initio (from the beginning) approach

NGRA Case Study - 1

TIER 0: Identify use scenario and collect existing information



Move to Tier 1

- Leave on lotion - twice daily
- 8 g/day
- Ingredient at 0.01%

- MW = 270 g/mol
- $LogP_{ow}$ = 3.04

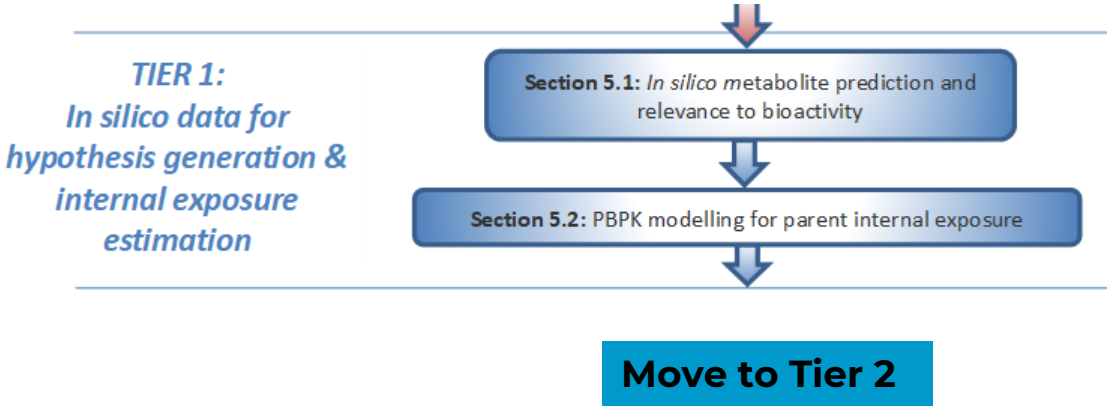
- Cramer Class III (2.3 µg/kg bw/day)
- 0.55 mg/day exposure
- 9.1 µg/kg bw/day

- CTD indicates ER pathway activity
- CERAPP and COMPARA strong binding to ER but not AR
- ToxCast data – ER activation, but much lower potency than 17β-estradiol

TTC not suitable; exposure-based waiving N/A

AR: androgen receptor; CERAPP: Collaborative Estrogen Receptor Activity Prediction Project; COMPARA: Collaborative Modeling Project for Androgen Receptor Activity; CTD: comparative toxicogenomics database; ER: estrogen receptor; MW: molecular weight; TTC: threshold of toxicological concern

NGRA Case Study - 1

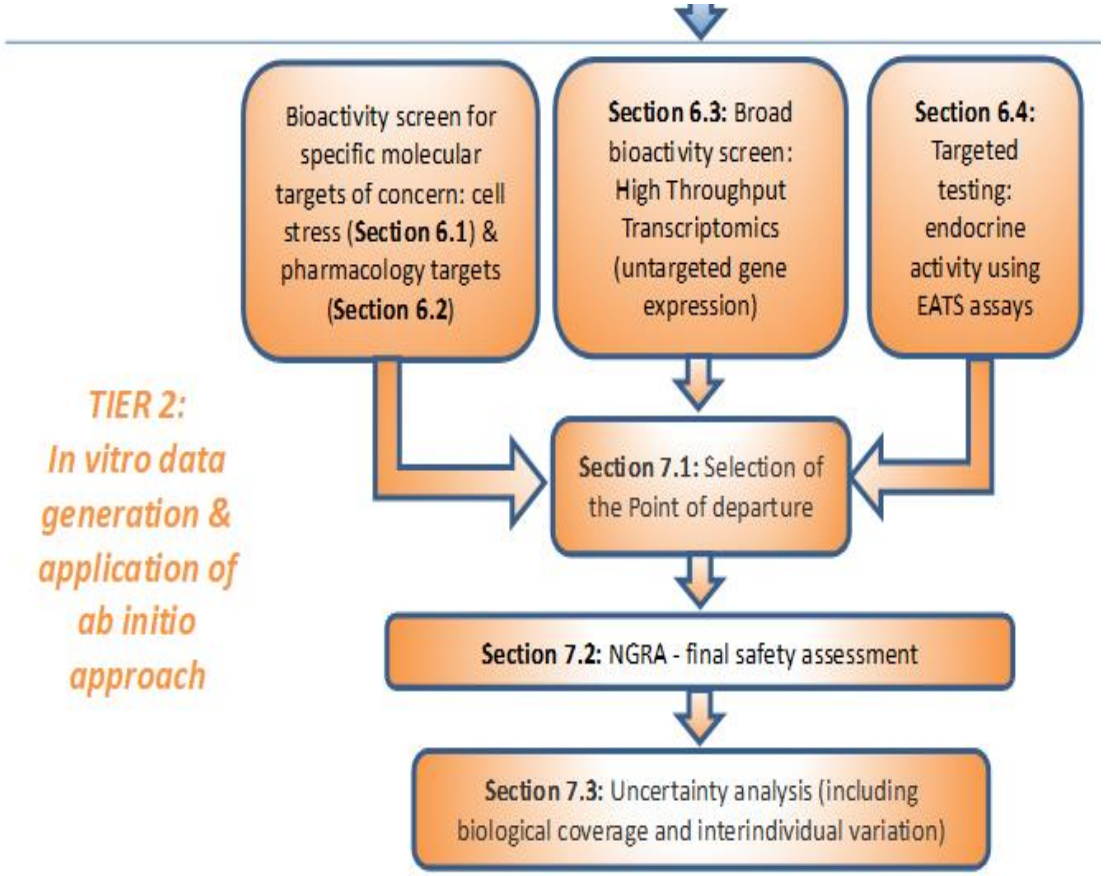


- 3 major metabolites (2 glucuronide and 1 sulfate conjugate) predicted in silico
  - Low probability of binding to AR, ER
  - Due to detoxification, not considered in Tier 2 bioassays.

- PBPK for total (0.27-061 nM) and unbound (0.006-0.1 nM) Cmax
- These values can be used to derive a bioactivity exposure ratio for final safety assessment

Cmax: maximum (or peak) serum concentration that a substance achieves in a specified compartment or test area of the body after the substance has been administered and before the administration of a second dose; PBPK: physiologically based pharmacokinetic modeling

NGRA Case Study - 1



- 8 cell stress pathways covered by 13 HCS assays
  - PODs > 10 µM
- Pharmacology profiling – 80 targets
  - ~10% were hits (i.e., > 50% inhibition/stimulation)
  - ER most potently affected hits
- HTT - lowest median BMD for estrogen-dependent gene expression = 0.11 µM
- EATS assays - lowest POD was ERα + S9 (LOEC = 5.2 nM)

- POD based on weight of evidence = 1.73 nM for estrogenic effects

- BER = 2.8 (1.73/0.61) to 288 (1.73/0.006)

- Moderate to high confidence associated with the data types used
- Low levels of uncertainty related to correlation of receptor binding to activity and extent of biological coverage

BER: bioactivity exposure ration; BMD: benchmark dose; EATS: estrogen, androgen, thyroid, steroidogenesis; HCS: high content screening; HTT: high throughput transcriptomics; LOEC: lowest observable effective concentration; POD: point of departure

Overall Safety Assessment

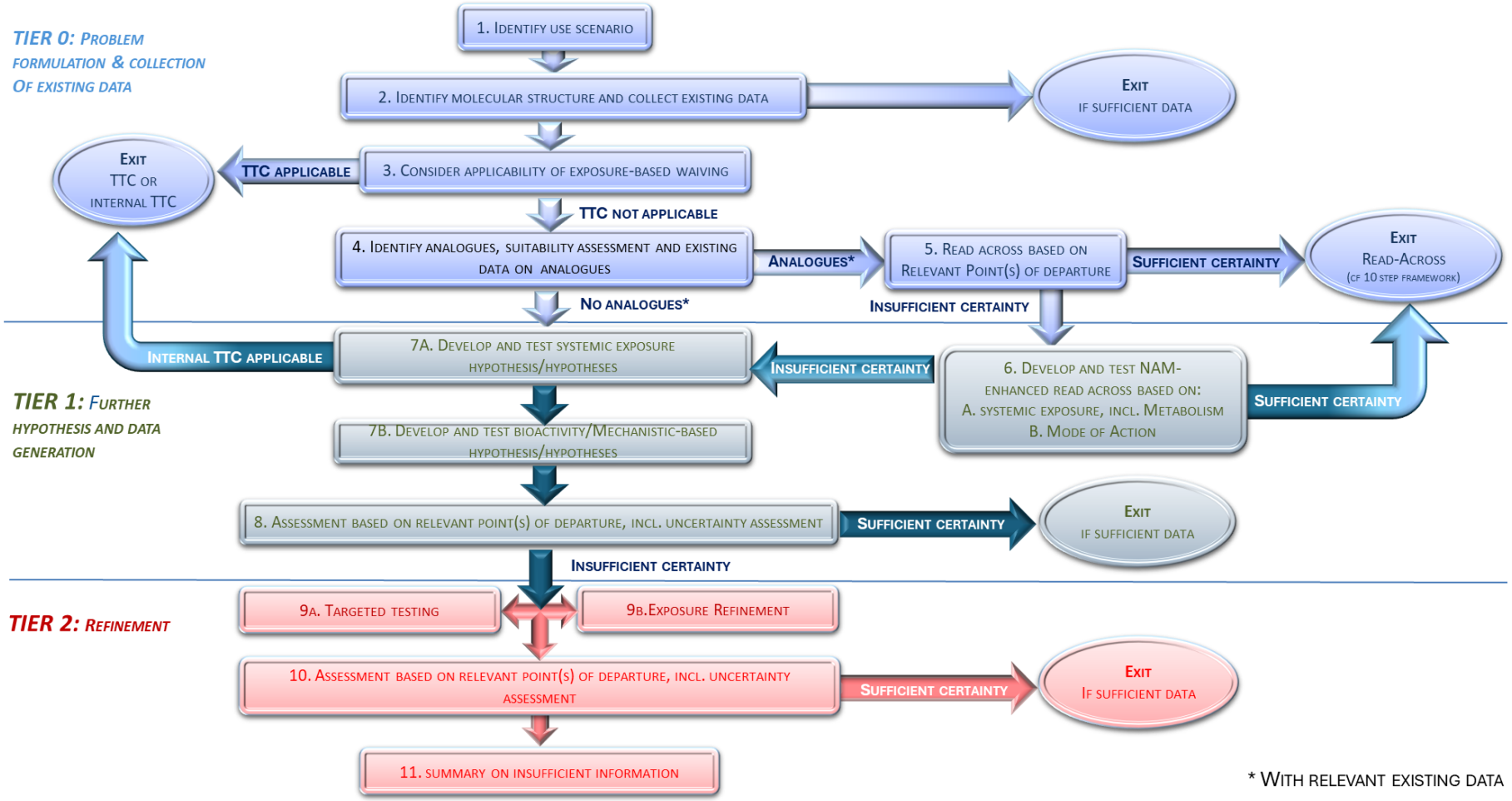
- BERs 2.8-288
  - In vitro bioactivity not observed under the consumer-relevant exposure scenario.

- When the **results of the NGRA** were **compared** with the conclusion from a **safety assessment using traditional methods**, the NAM-based safety assessment appears to be **sufficiently protective** for the consumer



NGRA Case Study – 2: read across

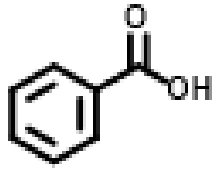
- **Integration of in silico, in vivo and in vitro data to enhance the RAX assessments of low systemic toxicity chemicals**



NGRA Case Study - 2

1. IDENTIFY USE SCENARIO

2. IDENTIFY MOLECULAR STRUCTURE AND COLLECT EXISTING DATA



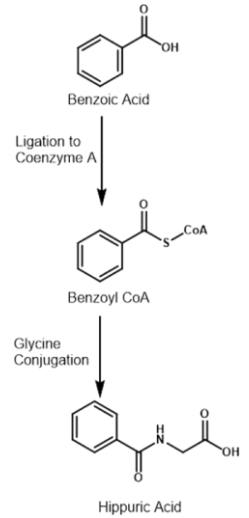
3. CONSIDER APPLICABILITY OF EXPOSURE-BASED WAIVING

TTC NOT APPLICABLE

4. IDENTIFY ANALOGUES, SUITABILITY ASSESSMENT AND EXISTING DATA ON ANALOGUES

ANALOGUES*

- Benzoic acid (BA), widely used as a preservative in cosmetics and food products
- Low toxicity - Free-standing NOEL of 500 mg/kg/day, from a 4-gen. dietary rat study (Kieckebusch and Lang, 1960)
- Biotransformation occurs in liver and kidney via formation of benzoyl CoA, a substrate for glycine conjugation to form hippuric acid



- Can a category of benzoates be defined, and the same low toxicity be assured using NAM-based read across approaches?

Analog Identification

4. IDENTIFY ANALOGUES, SUITABILITY ASSESSMENT AND EXISTING DATA ON ANALOGUES → **ANALOGUES***

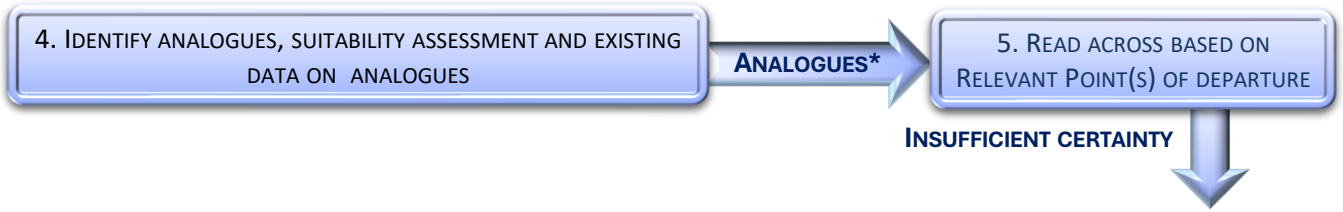
- Matched Molecular Pair approach (Lester & Yan, 2021) to define potential analogues and assign tentative suitability ratings
  - Considers suitability based on similarity in (sub)structure, metabolism, reactivity, and physicochemical properties (Blackburn et al., 2011; Wu et al., 2010)

- 24 Chemicals considered*
  - S = 8; SWI = 8; NS = 8

Chemical	Structure	CAS	MW g/mol	LogP	pKa (Acid) (charge at pH 7)	Legacy in vivo data available ?	Analogue Rating Provisional
Benzoic acid		65-85-0	122.12	1.874	4.202 (neg)	Yes	Target
Sodium benzoate		532-32-1	144.103	1.874	4.202 (neg)	No	Suitable
Potassium benzoate		582-25-2	160.212	1.874	4.202 (neg)	No	Suitable
Calcium di-benzoate		2090-05-3	282.305	1.874	4.202 (neg)	Yes	Suitable
Magnesium di-benzoate		553-70-8	266.532	1.874	4.202 (neg)	No	Suitable
Ammonium benzoate		1863-63-4	139.152	1.874	4.202 (neg)	No	Suitable
p-Toluic acid		99-94-5	136.148	2.349	4.372 (neg)	Yes	Suitable
m-Toluic acid		99-04-7	136.148	2.349	4.272 (neg)	Yes	Suitable
o-Toluic acid		118-90-1	136.148	2.349	3.947 (neg)	No	Suitable
3,5-dimethyl benzoic acid		499-06-9	150.175	3.251	4.245	No	Suitable with Interpretation
3,4-dimethyl benzoic acid		619-04-5	150.175	2.742	4.442 (neg)	No	Suitable with Interpretation
2,3-dimethyl benzoic acid		603-79-2	150.175	2.742	3.556 (neg)	No	Suitable with Interpretation
2,4,5-trimethyl benzoic acid		528-90-3	164.201	3.251	3.846 (neg)	No	Suitable with Interpretation
Cumic acid		536-66-3	164.201	2.618	4.35 (neg)	No	Suitable with Interpretation
2,4,6-Trimethyl benzoic acid		480-63-7	164.201	3.706	3.958 (neg)	Yes	Suitable with Interpretation
4-Ethyl benzoic acid		619-64-7	150.175	3.158	4.352 (neg)	No	Suitable with Interpretation
2,6-Dimethyl benzoic acid		632-46-2	150.175	2.742	4.442 (neg)	No	Suitable with Interpretation
4-Hydroxy benzoic acid		99-96-7	138.121	1.6	4.572 (neg)	Yes	Not Suitable
Salicylic acid		69-72-7	138.121	2.371	4.006 (neg)	Yes	Not Suitable
4-tert-Butyl benzoic acid		98-73-7	178.228	3.117	4.082 (neg)	Yes	Not Suitable
Propylparaben		94-13-3	180.200	2.809	(neutral)	Yes	Not Suitable
Butylparaben		94-26-8	194.227	3.125	(neutral)	Yes	Not Suitable
Isopropylparaben		4191-73-5	180.200	3.183	(neutral)	No	Not Suitable
Methylparaben		99-76-3	152.147	2.143	(neutral)	Yes	Not Suitable
Ethylparaben		120-47-8	166.174	2.529	(neutral)	Yes	Not Suitable

Suitable
Suitable with Interpretation
Not suitable

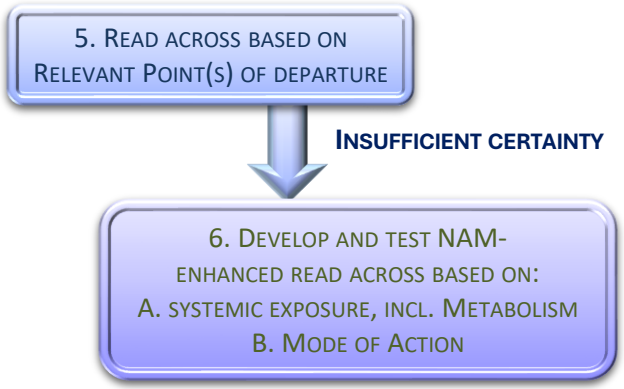
Analog Data Collection



- Discordant in vivo toxicity data for some suitable analogues
  - Oral **gavage** studies demonstrate toxicity at administered doses lower than **dietary** studies for BA

		Legacy in vivo	NOEL	Findings
Benzoic Acid		**dietary** multi-generation rat study **dietary** EOGRT study	500 mg/kg/day 1000 mg/kg/day	free standing highest dose tested
Salts	Sodium	RAX to benzoic acid (JECFA, SCCP, EFSA)		
	Potassium	RAX to benzoic acid (JECFA, ECHA, EFSA)		
	Calcium	**gavage** 28-day repeated dose rat study	300 mg/kg/day	1000 mg/kg/day - ↓ bw in male rats exhibited reduced body weights, ↓ food consumption, ↑ liver and kidney weights, histological changes, ↓ T4 levels, and ↑ bile acids. Female rats at the high dose showed ↑ kidney weights and liver histological findings.
	Magnesium	ND		
	Ammonium	ND		
Single methyl substituted	p-toluic acid	**gavage** 28-day repeated dose rat study	1000 mg/kg/day	
	m-toluic acid	**gavage** 28-day repeated dose rat study	100 mg/kg/day	1000 mg/kg/day - females experienced ↑ absolute and relative liver weights and periportal hepatocellular vacuolar degeneration, with similar but less severe effects noted in 300 mg/kg/day dose group.
	o-toluic acid	ND		

A. Systemic Exposure

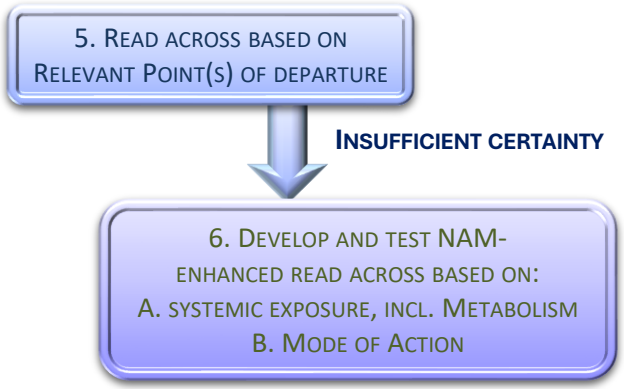


- Bioavailability
  - Dermal penetration of benzoic acid is moderate-high
  - Similar physicochemical properties for innocuous salts and small alkyl derivatives indicative of similar bioavailability

Chemical	Parent (µM) at 0 min	% Remaining at 240 min	$t_{1/2}$ (min)	$CL_{int, in\ vitro}$ (µL/min/10^6 cells)	Glycine Conjugate (µM)	Glucuronide (Peak area)	Oxidation (Peak area)	Oxidative Desaturation (Peak area)
Benzoic Acid	50	2.9	44.4	19.5	60	ND	ND	ND
Sodium Benzoate (S)	50	2.9	44.5	19.5	60	ND	ND	ND
m-Toluic Acid (S)	50	0.7	32.6	26.5	64	trace	ND	ND
p-Toluic Acid (S)	51	6.2	24.5	35.3	44	ND	ND	ND
o-Toluic Acid (SI)	51	72.9	> 180	1.7	10	4214	ND	ND
Cumic Acid (SI)	64	33.7	164.9	5.3	0	37727	462861	13288
2,4,6-Trimethylbenzoic acid (SI)	25	96.5	> 240	0.0	0	15605	4972	ND

- Metabolism (newly generated data)
  - Human hepatocytes show importance of glycine conjugation for BA, SB, m- and p-toluic acids

A. Systemic Exposure

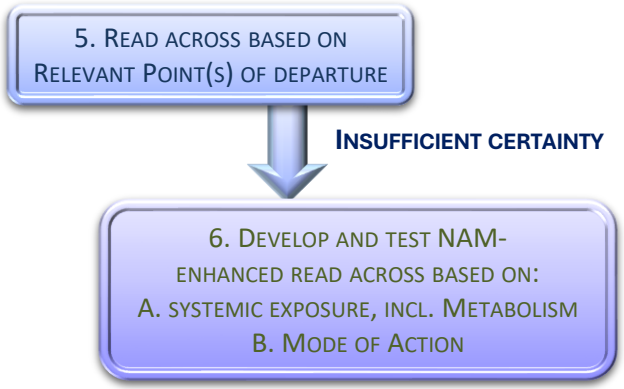


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- Metabolism (newly generated data)
  - Human hepatocytes show importance of glycine conjugation for BA, SB, m- and p-toluic acids
  - o-toluic acid slower metabolism, → reassign rating of ‘Suitable” to “Suitable with Interpretation”
  - Cumic acid undergoes glucuronidation and hydroxylation, while 2,4,6-trimethoxybenzoic acid shows little parent loss → reassign rating of ‘Suitable with Interpretation” to “Not Suitable”

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B. Mode of Action

5. Read across based on Relevant Point(s) of departure

Insufficient certainty

6. Develop and test NAM-enhanced read across based on:
A. systemic exposure, incl. Metabolism
B. Mode of Action

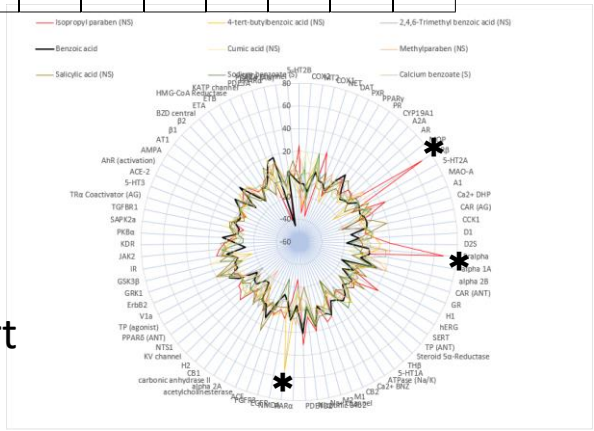
- Patchwork of data in Toxcast points to low # hits

		# Toxcast Hits	# Endpoints	% Endpoint Overlap With Benzoic Acid	Hits with Flags
Benzoic Acid		5	839	100	5 (100%)
Salts	Sodium (S)	0	627	66	0 (0%)
	Potassium (S)	1	218	26	1 (100%)
	Calcium (S)	No data	No data	No data	No data
	Magnesium (S)	0	318	37	0 (0%)
	Ammonium (S)	No data	No data	No data	No data
Single methyl substituted	p-toluic acid (S)	4	218	26	4 (100%)
	m-toluic acid (S)	6	290	34	4 (66%)
	o-toluic acid (SWI)	3	218	26	0 (0%)

- Transcriptomics data (≤500µM in 4 cell types) builds confidence in low DEGs for suitable analogues
  - *Ca salt showed larger DEGs, indicative of highly acidic local environment
  - Some "not suitable" analogues show gene dysregulation (e.g., isopropyl paraben)

Alkyl benzoic acid	Benzoic Acid (DEGs)	Benzoate salts (DEGs)	Methyl substituted benzoic acids (DEGs)			Alkyl benzoic acid (DEGs)		Hydroxy benzoic acid (DEGs)
	Benzoic Acid (Source)	Na^+, K^+, Mg^{2+}, NH_4^+ Ca^{2+} (S)	p-toluic acid (S)	m-toluic acid (S)	o-toluic acid (SI)	2,4,6-trimethyl benzoic acid (NS)	4-tertbutyl benzoic acid (NS)	Isopropyl paraben (NS)
	MCF7-0	MCF7-0	MCF7-0 HepG2-0	MCF7-0 HepG2-0	MCF7-0 HepG2-0	MCF7-1 HepG2-0	MCF7-0 HepG2-0	MCF7-1847
	HepG2-1	HepG2-4/0 /1 /0[a]	A459-0	A459-0	A459-0	A459-0	A459-0	HepG2-1926
	A459-0	A459-0	iCAR- 0	iCAR- 0	iCAR- 0	iCAR- 0	iCAR- 1	A459-1922
	iCAR-1	iCAR-0						iCAR-1476
		Ca^{2+}: ✱ MCF7-0 HepG2-544 A459-29 iCAR-46						

- Pharmacology profiling (10µM) supports no specific target for suitable analogues
  - *Some NS analogues show activity (e.g., isopropyl paraben (ERα and ERβ) and 4-tert butylbenzoic acid (NMDA))



DEG: Differentially expressed genes

Describing Similarity of Analogs

- Fingerprints designed following the qSIM approach described by Lester et al. (2023)
  - Tanimoto is used to compute a similarity score based on how the fingerprints overlap

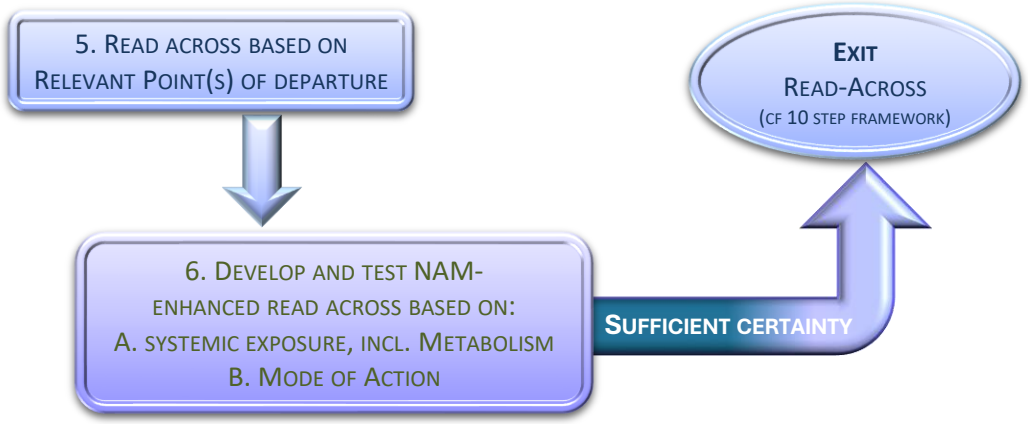
  - **Reactivity**
    - Reactivity information from the NAM data (TxG and APPP) was combined with structural alerts (DEREK and DART DT)
  - **Physicochemical properties**
    - in silico predictions
  - **Metabolism**
    - Biotransformation data

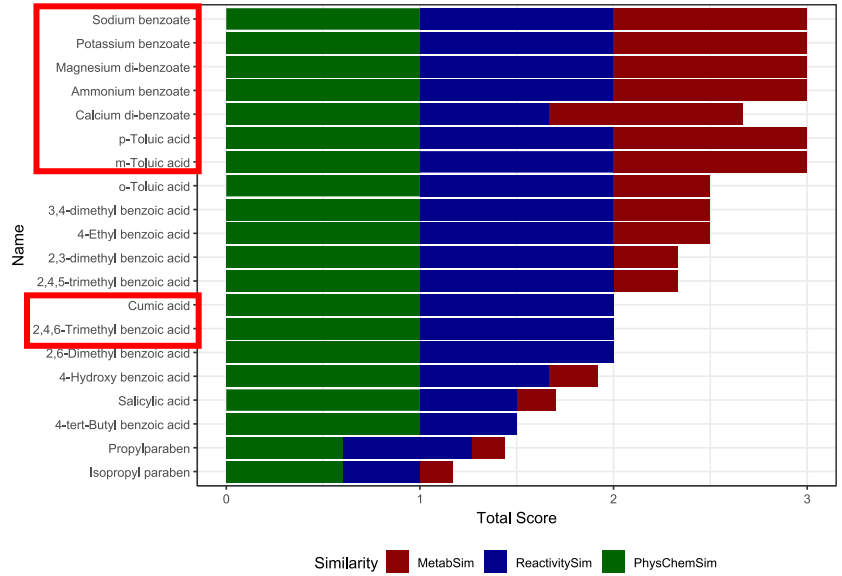
Alerts and Reactivity	BA	2090-05-3	99-94-5	99-04-7	118-90-1	619-64-7	480-63-7	536-66-3	98-73-7	69-72-7	99-96-7	4191-73-5
Mutagenicity in vitro bacterium_INACTIVE	1	1	1	1	1	1	1	1	1	1	1	1
Skin sensitisation mammal_NON-SENSITISER	1	1	1	1	1	1	1	1	1	1	1	1
PG Custom Skin sensitisation Alert 1 name_Phenol	0	0	0	0	0	0	0	0	0	1	1	0
Hepatotoxicity mammal_551	0	0	0	0	0	0	0	0	0	1	0	0
Nephrotoxicity mammal_817	0	0	0	0	0	0	0	0	0	1	0	0
Maps DART Structure_2b-3-3a	0	0	0	0	0	0	0	0	0	0	0	1
Maps DART Structure_9b-2	0	0	0	0	0	0	0	0	1	0	0	0
Transcriptional Profiling (> 4 genes affected)	0	1	0	0	0	0	0	0	0	-	-	1
Pharmacological Profiling (1 or more positive assay)	0	0	-	-	-	-	0	0	1	0	-	1
Reactivity Similarity Score	**SOI**	**0.67**	**1**	**1**	**1**	**1**	**1**	**0.67**	**0.50**	**0.50**	**0.67**	**0.40**

PhysChem Similarity Keys	BA	99-94-5	99-04-7	118-90-1	619-64-7	480-63-7	536-66-3	98-73-7	69-72-7	99-96-7	4191-73-5
MWtBin-2	1	1	1	1	1	1	1	1	1	1	1
ΔLogP Bin-1	1	1	1	1	1	1	1	1	1	1	1
Charge – Neg	1	1	1	1	1	1	1	1	1	1	0
Charge – Neutral	0	0	0	0	0	0	0	0	0	0	1
VOC	0	0	0	0	0	0	0	0	0	0	0
PhysChem Similarity Score	**SOI**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0.50**

Metabolism Keys	BA	532-32-1	99-94-5	99-04-7	118-90-1	619-64-7	480-63-7	536-66-3	98-73-7	69-72-7	99-96-7	4191-73-5
glycination_(aromatic_carboxyl)	1	1	1	1	1	1	0	0	0	1	1	1
ortho_substituted_carboxyl	0	0	0	0	1	0	1	0	0	1	0	0
O-glucuronidation_(aromatic_carboxyl)	0	0	0	0	0	1	0	1	1	1	1	1
O-glucuronidation_(aromatic_hydroxyl)	0	0	0	0	0	0	0	0	0	1	1	1
aliphatic_hydroxylation_(alkyl)	0	0	0	0	0	0	0	1	1	0	0	0
aromatic_hydroxylation	0	0	0	0	0	0	0	1	0	1	0	1
aliphatic_desaturation	0	0	0	0	0	0	0	1	0	0	0	0
hydrolysis_(ester)	0	0	0	0	0	0	0	0	0	0	0	1
sulfation_(aromatic_hydroxyl)	0	0	0	0	0	0	0	0	0	0	1	1
Metabolism Similarity Score	**SOI**	**1**	**1**	**1**	**0.5**	**0.5**	**0**	**0**	**0**	**0.20**	**0.25**	**0.17**

Quantitative Similarity Assessment





- Quantitative similarity approach (qSIM) to produce a TotalScore of suitability and define a category
  - **TotalScore**= total metabolism similarity + alert similarity + physicochemical similarity

- Suitable analogues in the category have the highest TotalScores of 3 and include Na, K, Mg and NH4 benzoate, p-toluic acid and m-toluic acid plus Ca benzoate (with a score of 2.67)

- For this case, metabolism had the greatest impact on similarity scores
  - A lack of structural alerts was generally supported by in vitro NAM data
  - Differences in reactivity were observed for NS analogues outside the read across category

NGRA Case Studies: Key Learnings

- The **collective experience**, along with the various hypotheses explored and methodology used in the different cases, has led to **increased confidence** in an exposure-led, mode of action driven framework for systemic toxicity assessment
- Read across allows the use of analogues to **fill data gaps** and aid in supporting mode of action and potency assessments
- A combination of non-targeted broad testing approaches combined with targeted assays provided measures of bioactivity which appear to be **at least as protective as traditional approaches**.
- **Conservatism** in the approach **could be refined** by employing higher tier testing to distinguish bioactivity from adversity, and to test the biological and chemical coverage of the NAMs.





Thank you, Microsoft Copilot

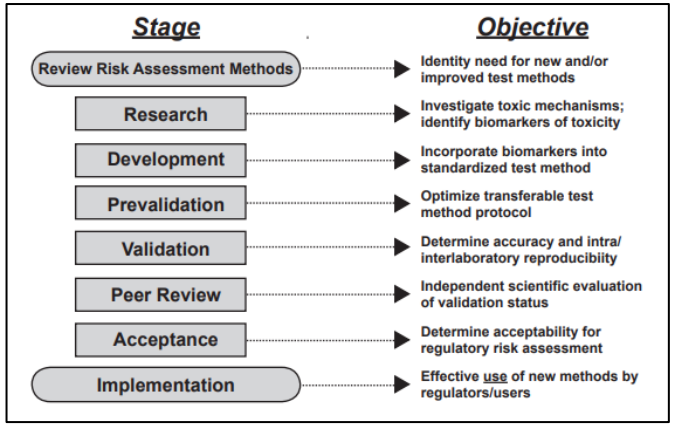
Test Method Validation – A Historical Perspective

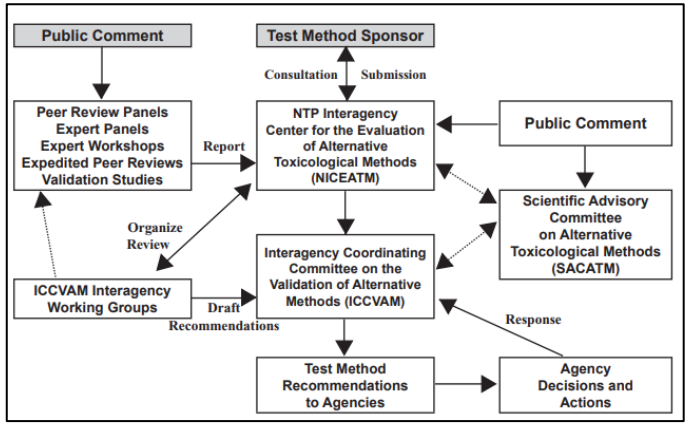




Historical Test Method Validation Process

Historical Test Method Evaluation Process





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Many NAMs are Internationally Accepted

Toxicity endpoint	Available In silico and Read Across	Validated In vitro Methods	
Skin corrosion/ irritation			
Eye corrosion/ irritation			
Skin sensitization			
Phototoxicity			
Toxicokinetics		A	DME
Repeated dose toxicity			
Reproductive toxicity			
Genotoxicity			
Carcinogenicity		CTA	
Endocrine activity		EA	ED

'Simple' replacement by a limited number of NAMs

A: absorption; CTA: cell transformation assay; DME: distribution, metabolism, excretion;
EA: endocrine activity; ED: endocrine disruption

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Table courtesy of Dr. Vera Rogiers

Reproducibility of Reference Test Methods

Prior type	1	2A	2B	NC
1	73%	16.1%	0.4%	10.4%
2A	4.2%	32.9%	3.5%	59.4%
2B	0.2%	4%	15.5%	80.2%
NC	1.1%	3.5%	1.5%	93.9%

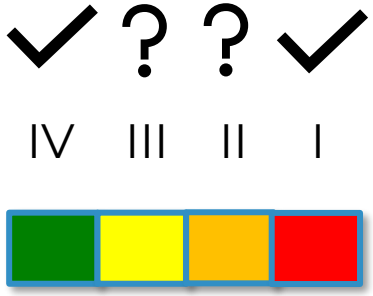
Ocular; Luechtefeld et al. 2016 ALTEX

Prior type	COR	II	III	IV
COR	57.9%	34.5%	6.2%	1.3%
II	5.7%	66.5%	27.5%	0.4%
III	0.5%	11.0%	79.8%	8.7%
IV	0.1%	0.6%	44.7%	54.6%

Acute oral; Karmaus et al. 2022 Tox Sci

Prior type	COR	II	III	IV
COR	86.3%	4.2%	7.1%	2.5%
II	14.1%	44.9%	20.5%	20.5%
III	6.9%	5.2%	53.6%	34.3%
IV	0.9%	2.0%	9.1%	88.0%

Dermal; Rooney et al. 2021 Reg Tox Pharm

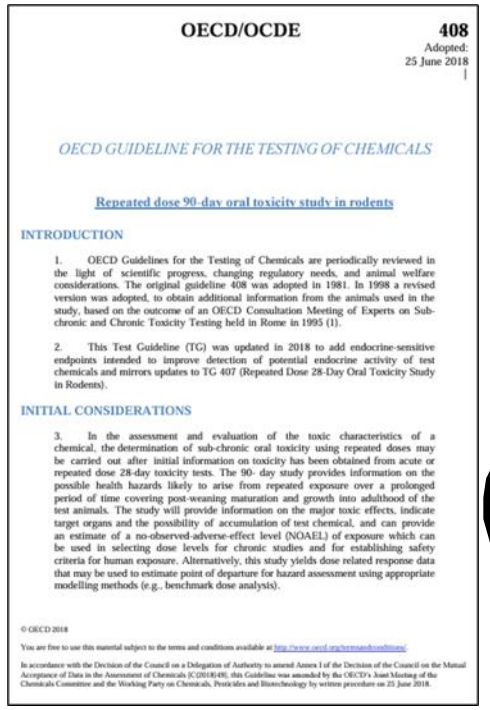






Replacing Animal Test Methods: What is the Actual Goal?

Breadth of Biological Coverage



OECD/OCDE 408
Adopted: 25 June 2018

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Repeated dose 90-day oral toxicity study in rodents

INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress, changing regulatory needs, and animal welfare considerations. The original guideline 408 was adopted in 1981. In 1998 a revised version was adopted, to obtain additional information from the animals used in the study, based on the outcome of an OECD Consultation Meeting of Experts on Sub-chronic and Chronic Toxicity Testing held in Rome in 1995 (1).

2. This Test Guideline (TG) was updated in 2018 to add endocrine-sensitive endpoints intended to improve detection of potential endocrine activity of test chemicals and mirrors updates to TG 407 (Repeated Dose 28-Day Oral Toxicity Study in Rodents).

INITIAL CONSIDERATIONS

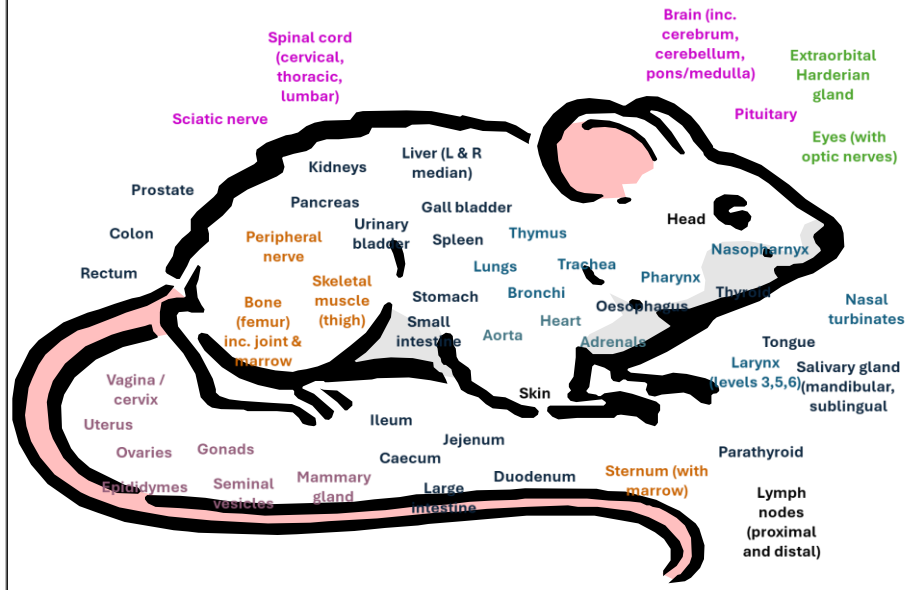
3. In the assessment and evaluation of the toxic characteristics of a chemical, the determination of sub-chronic oral toxicity using repeated doses may be carried out after initial information on toxicity has been obtained from acute or repeated dose 28-day toxicity tests. The 90- day study provides information on the possible health hazards likely to arise from repeated exposure over a prolonged period of time covering post-weaning maturation and growth into adulthood of the test animals. The study will provide information on the major toxic effects, indicate target organs and the possibility of accumulation of test chemical, and can provide an estimate of a no-observed-adverse-effect level (NOAEL) of exposure which can be used in selecting dose levels for chronic studies and for establishing safety criteria for human exposure. Alternatively, this study yields dose related response data that may be used to estimate point of departure for hazard assessment using appropriate modelling methods (e.g., benchmark dose analysis).

© OECD 2018

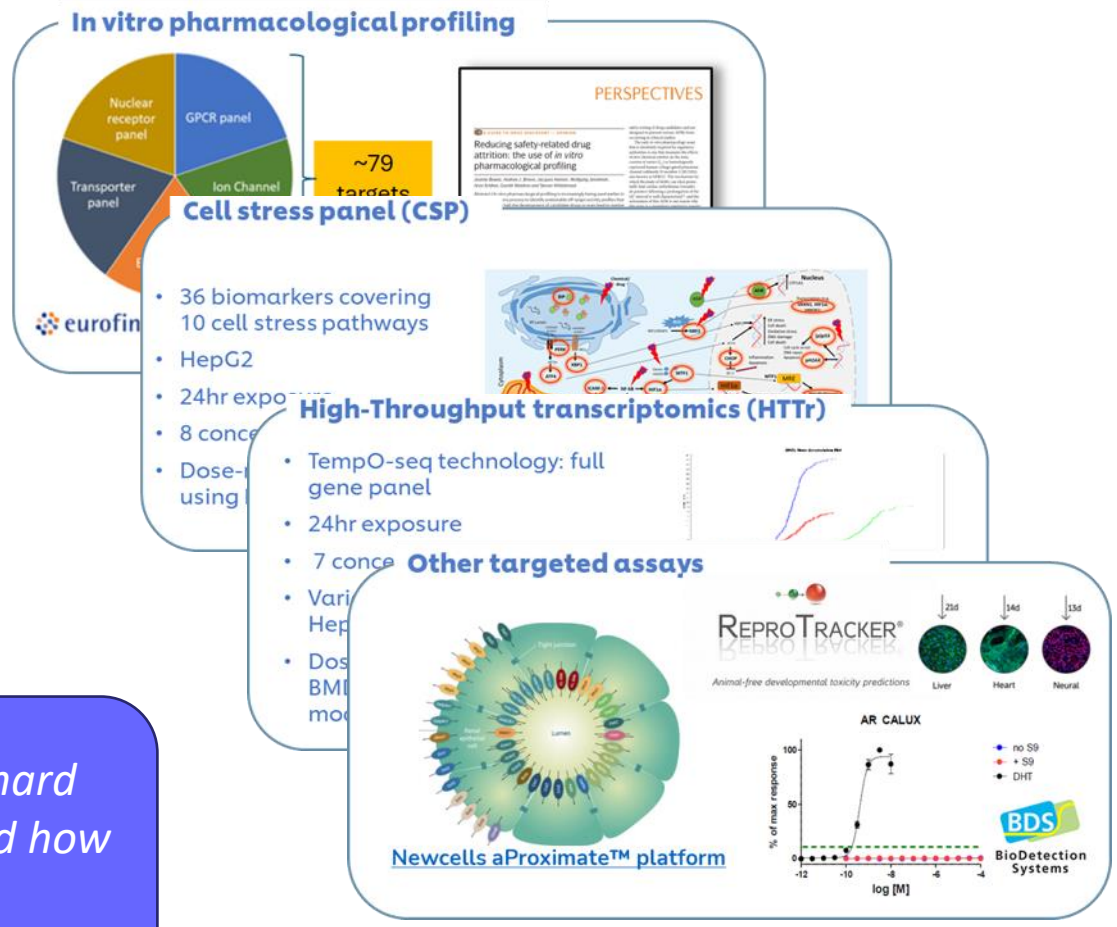
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In accordance with the Decision of the Council on a Delegation of Authority to amend Annex I of the Decision of the Council on the Mutual Acceptance of Data in the Assessment of Chemicals [C(2018)49], this Guideline was amended by the OECD's Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology by written procedure on 25 June 2018.

Historical: animal *in vivo*



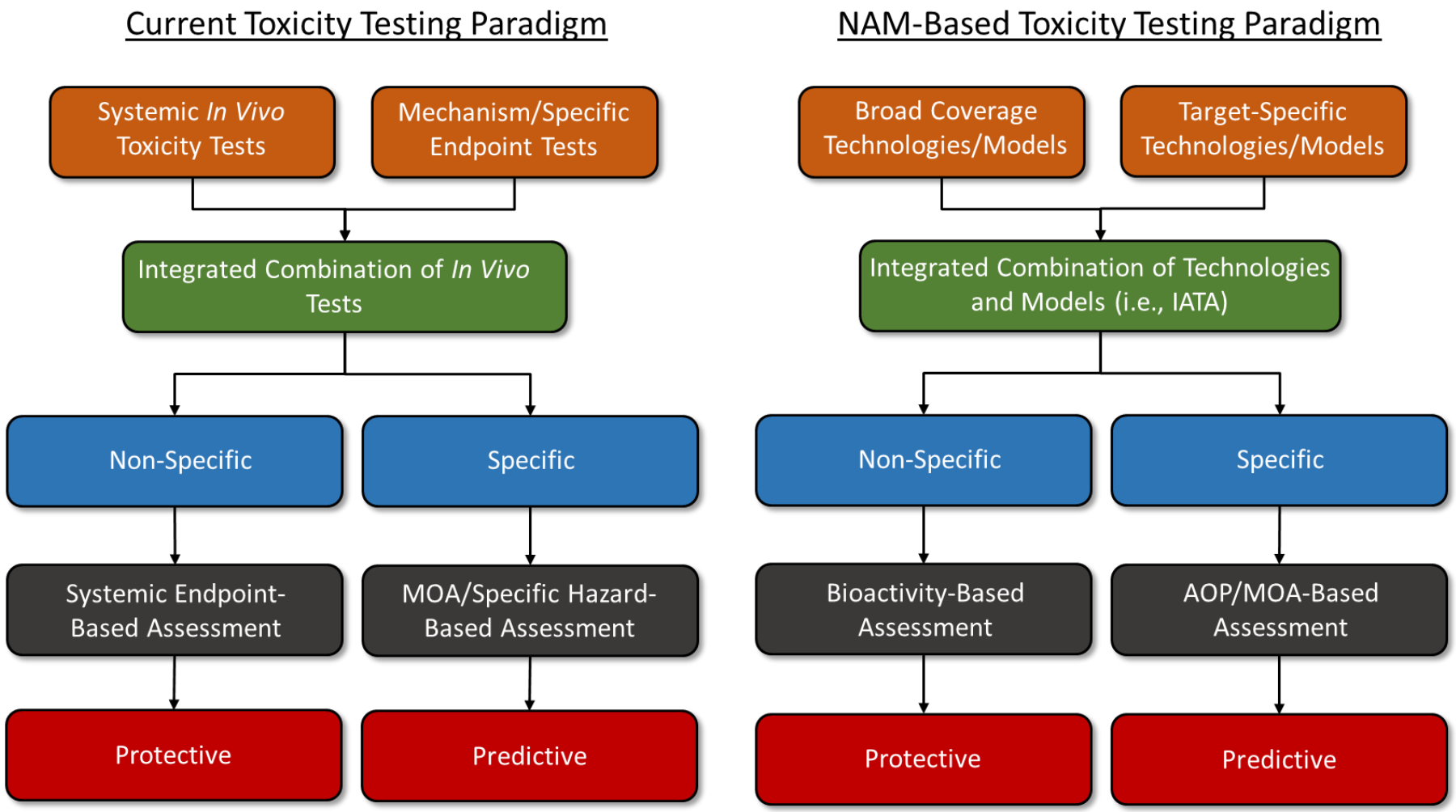
New: human-derived *in vitro*



> *"It's easy to say that models are wrong. The hard part is figuring out which models are useful and how wrong they are."*
> (George Box)

Material from Matt Dent (Unilever)

Predictive and Protective approaches



Browne, Paul Friedman, Boekelheide, Thomas (2024)

The New Era of ‘Acceptance’

From

- Centralized
  (efforts by validation centers)
- One Size Fits All
- Binary Status (Validated / Not)
- Stand Alone

TRANSITION



Towards

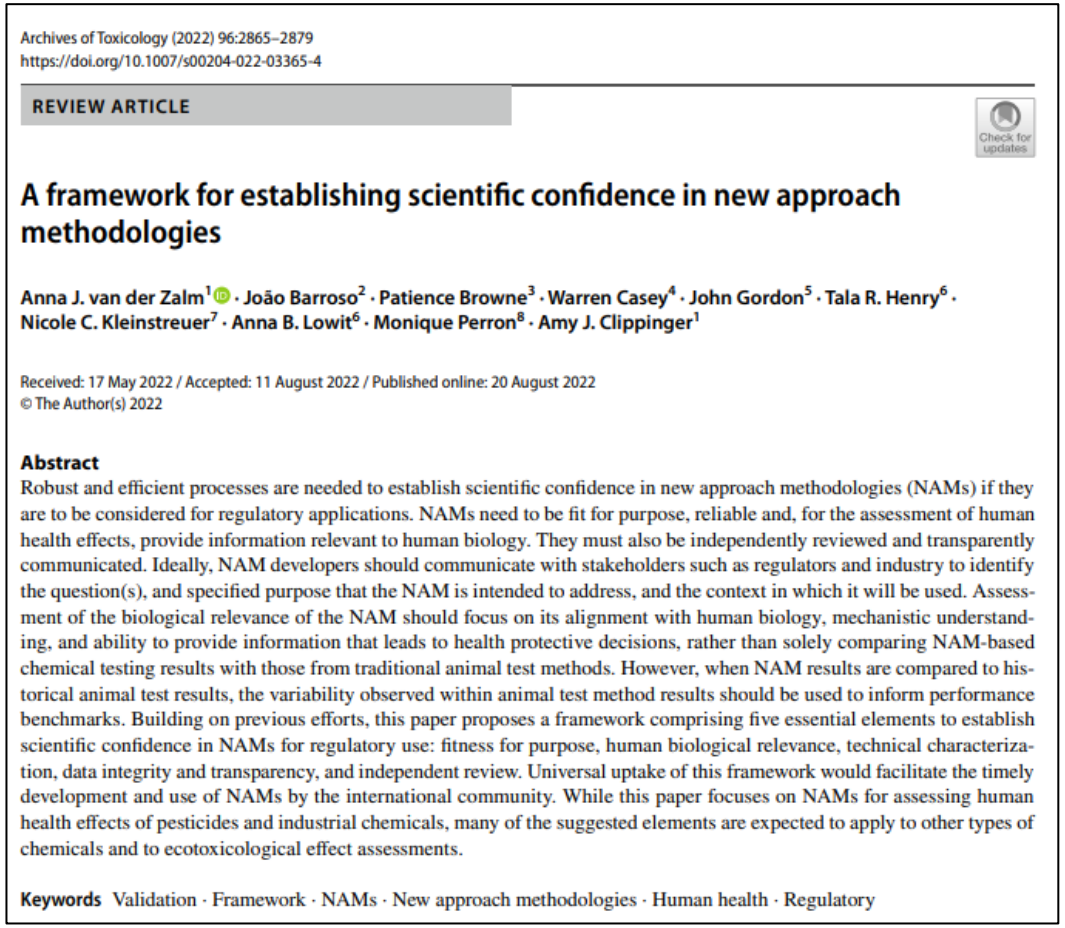
- Decentralized
  (End Users)
- Fit for Purpose
- Evolving Confidence
- Integrative

Acceptance of NAMs and NGRA beyond acute endpoints will rely on sustained collaboration with governments and regulators

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Modified from NICEATM

Purpose = Context of Use



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https://doi.org/10.1007/s00204-022-03365-4

REVIEW ARTICLE

A framework for establishing scientific confidence in new approach methodologies

Anna J. van der Zalm[1] · João Barroso[2] · Patience Browne[3] · Warren Casey[4] · John Gordon[5] · Tala R. Henry[6] · Nicole C. Kleinstreuer[7] · Anna B. Lowit[6] · Monique Perron[8] · Amy J. Clippinger[1]

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Abstract
Robust and efficient processes are needed to establish scientific confidence in new approach methodologies (NAMs) if they are to be considered for regulatory applications. NAMs need to be fit for purpose, reliable and, for the assessment of human health effects, provide information relevant to human biology. They must also be independently reviewed and transparently communicated. Ideally, NAM developers should communicate with stakeholders such as regulators and industry to identify the question(s), and specified purpose that the NAM is intended to address, and the context in which it will be used. Assessment of the biological relevance of the NAM should focus on its alignment with human biology, mechanistic understanding, and ability to provide information that leads to health protective decisions, rather than solely comparing NAM-based chemical testing results with those from traditional animal test methods. However, when NAM results are compared to historical animal test results, the variability observed within animal test method results should be used to inform performance benchmarks. Building on previous efforts, this paper proposes a framework comprising five essential elements to establish scientific confidence in NAMs for regulatory use: fitness for purpose, human biological relevance, technical characterization, data integrity and transparency, and independent review. Universal uptake of this framework would facilitate the timely development and use of NAMs by the international community. While this paper focuses on NAMs for assessing human health effects of pesticides and industrial chemicals, many of the suggested elements are expected to apply to other types of chemicals and to ecotoxicological effect assessments.

Keywords Validation · Framework · NAMs · New approach methodologies · Human health · Regulatory

Fitness for Purpose

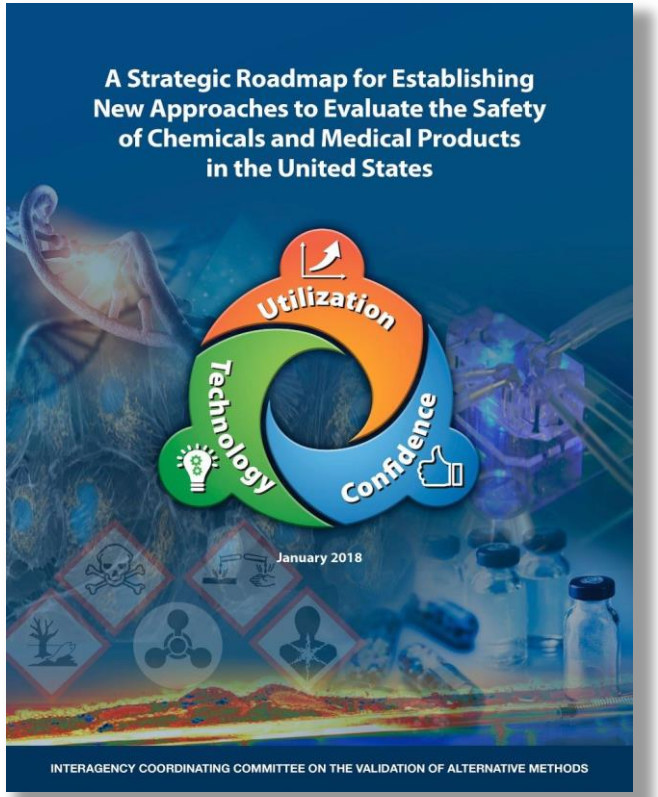
Which regulatory statutes are data from the NAM intended to comply with?
- U.S. TSCA
- EU REACH
- Other

How will the NAM be used?
- As a stand-alone assay
- As part of a defined approach
- As part of an integrated approach to testing and assessment or weight of evidence assessment

Is the information provided sufficient to address the regulatory endpoints of interest?
- Describe the relationship between the information measured by the NAM and the regulatory endpoints being addressed.
- Is the technical performance, including the level of uncertainty, acceptable?

What is the context in which the NAM is intended to be used?
- Preregulatory screening and prioritization
- Chemical grouping
- Hazard identification
- Quantitative risk assessment

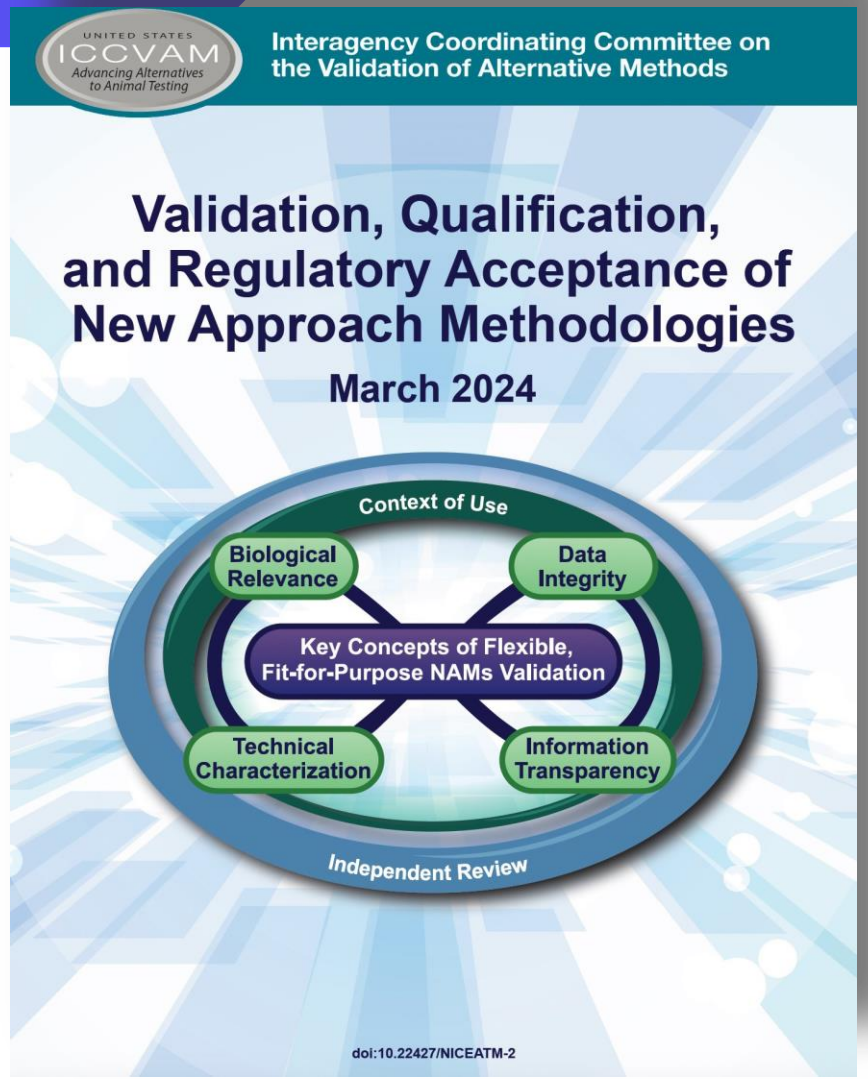
Establishing Confidence



https://ntp.niehs.nih.gov/go/natl-strategy

- Connect end users with the developers of alternative methods
- Establish new validation approaches that are more flexible and efficient
- Ensure adoption and use of new methods by both regulators and industry

Slide courtesy of Nicole C. Kleinstreuer, PhD



https://ntp.niehs.nih.gov/go/ICCVAM-submit

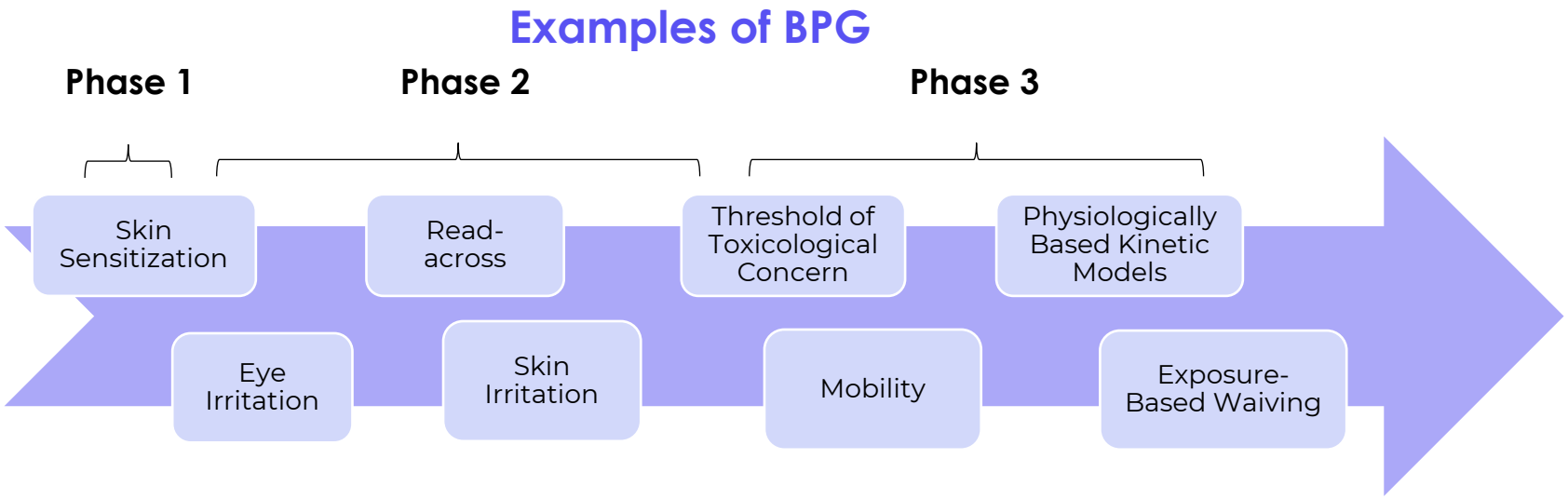
Increasing Uptake and Use of NAMs

ICCS Best Practice Guidance (BPG)

Best Practice Guidance (BPG)

BPG are being developed for safety assessors

- Developed with input from global subject matter experts among the ICCS membership
- Clear and concise step-by-step framework
- Beginning with problem formulation/decision context
- Suitable for global use



Purpose of ICCS BPG

- Provides a structured framework to help **safety assessors and regulatory scientists** integrate **NAMs** into safety assessments for cosmetic ingredients and products.
- **Bridges the gap** between advancing science and regulatory requirements by:
  - Offering **step-by-step workflows** for safety assessments.
  - Supporting **consistency, transparency, and reproducibility** in decision-making.
  - Offering **practical guidance for implementation** of NAMs and developed with expert consensus.
- Outlines recommended approaches that are **widely accepted** based on current knowledge and consensus.



Goals of ICCS BPG



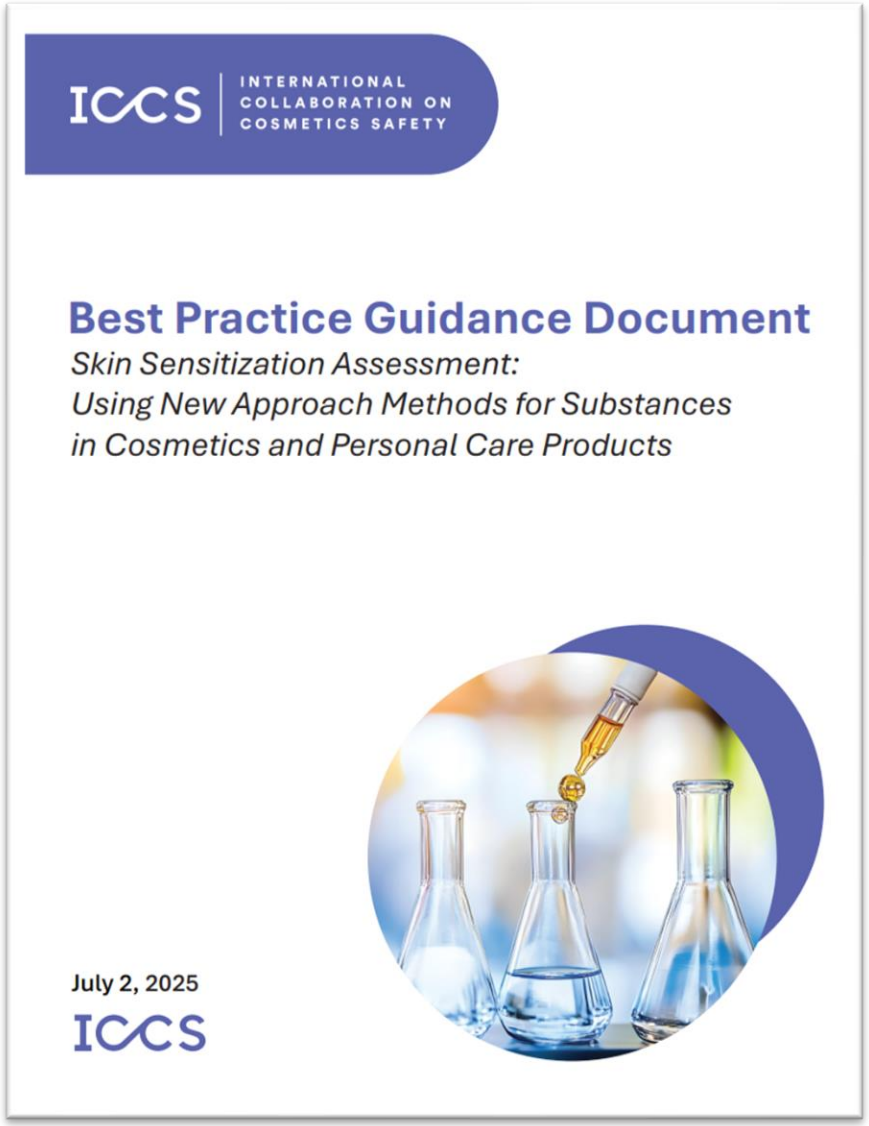
- **Enable broader adoption of NAMs** by providing practical, science-based guidance.
- Improve the **quality and robustness** of safety assessments.
- Facilitate **regulatory harmonization** by consensus-based approaches, aligned terminology, often leading to standard setting.
- Support **NGRA** principles through integrated, evidence-based frameworks

Intended Audience of BPG

- **Safety assessors** within the cosmetics and personal care industry.
- **Regulatory scientists** evaluating ingredient safety.
- **Toxicologists** developing, validating, or applying NAMs.
- **Academia, NGOs, and government agencies** engaged in risk assessment and policy development



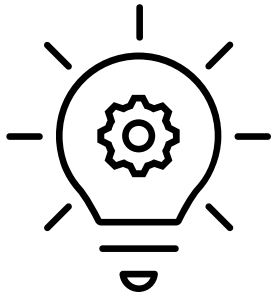
Skin Sensitization: The first ICCS BPG!



ICCS BEST PRACTICE GUIDANCE

https://www.iccs-cosmetics.org/education/best-practice-guidance/bpg-skin-sensitization-assessment-using-new-approach-methods

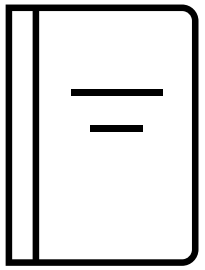
Skin Sensitization BPG



- Step-by-step workflow to guide through skin sensitization hazard and safety assessments without new animal tests
- Designed for regulatory use



- Streamlines decision-making
- Supports global harmonization



- Developed through consensus among ICCS members
- Incorporates insights from global subject matter experts



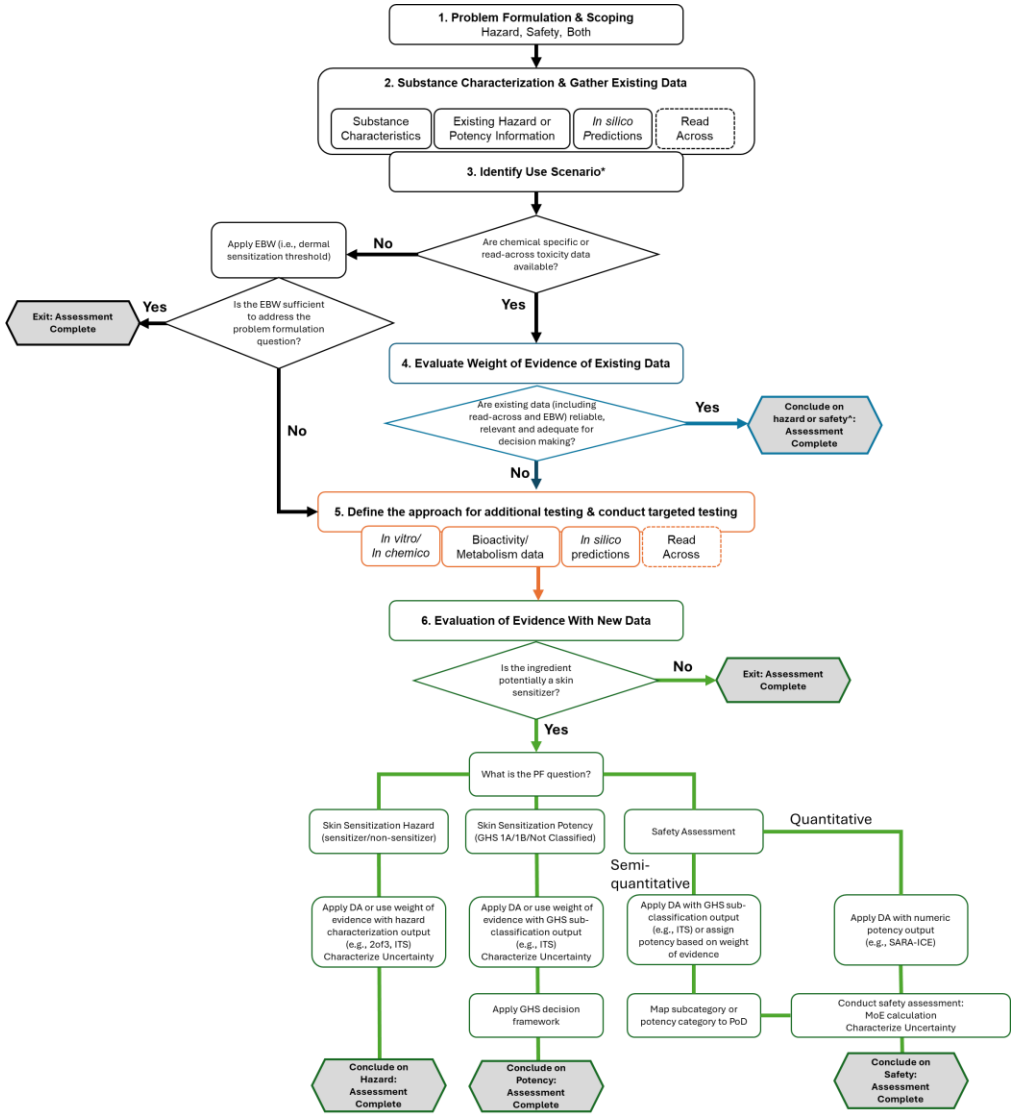
- For safety assessors familiar with traditional approaches, but less familiar with NAMs for SS

Building the Workflow



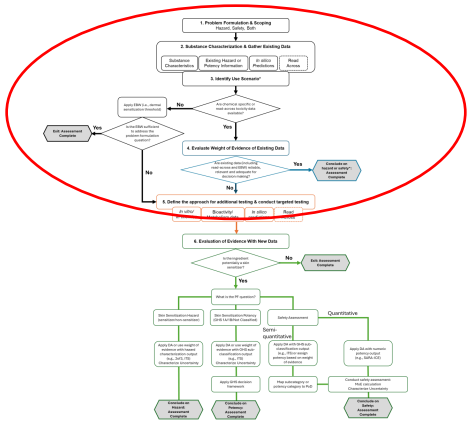
- If each lego brick is a step, put them in a logical order – what colour comes **first**? **Last**?

BPG Skin Sensitization Workflow



ICCS

BPG Skin Sensitization Workflow



1. Problem Formulation & Scoping
Hazard, Safety, Both

2. Substance Characterization & Gather Existing Data

Substance Characteristics | Existing Hazard or Potency Information | *In silico* Predictions | Read Across

3. Identify Use Scenario*

Are chemical specific or read-across toxicity data available?

No → Apply EBW (i.e., dermal sensitization threshold)

Yes

Is the EBW sufficient to address the problem formulation question?

Yes → Exit: Assessment Complete

No

4. Evaluate Weight of Evidence of Existing Data

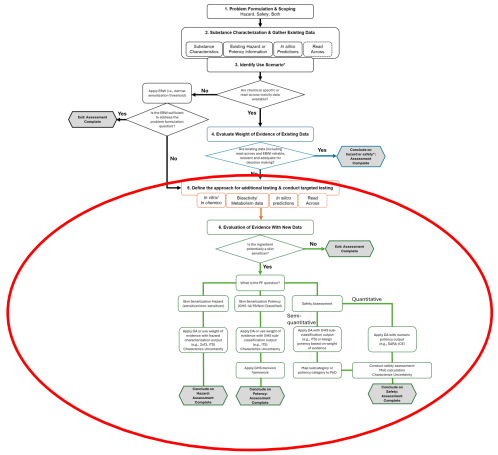
Are existing data (including read-across and EBW) reliable, relevant and adequate for decision making?

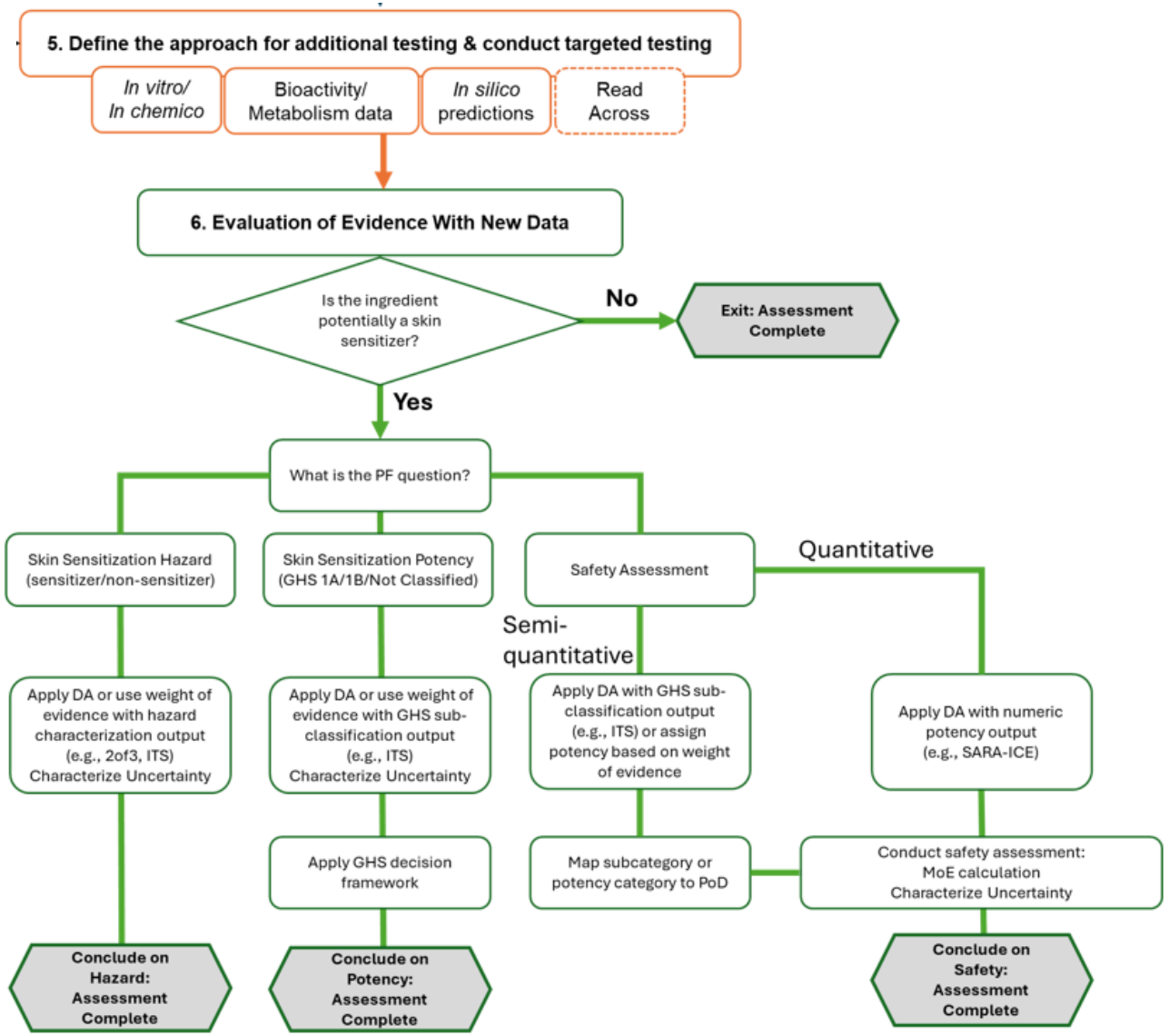
Yes → Conclude on hazard or safety^: Assessment Complete

No

5. Define the approach for additional testing & conduct targeted testing

BPG Skin Sensitization Workflow



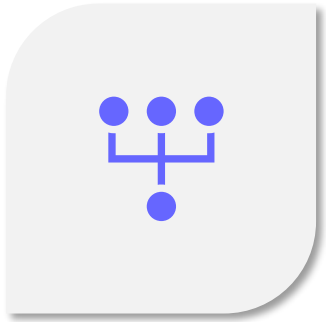


ICCS

NGRA: Take Home Messages



NGRA IS AN **EXPOSURE-LED**, HYPOTHESIS-DRIVEN **RISK ASSESSMENT** APPROACH THAT INTEGRATES *IN SILICO*, *IN CHEMICO* AND *IN VITRO* APPROACHES



NGRA CAN BE APPLIED USING A **TIERED FRAMEWORK** CONDUCTED AND DOCUMENTED AS DESCRIBED BY THE **9 ICCR PRINCIPLES**

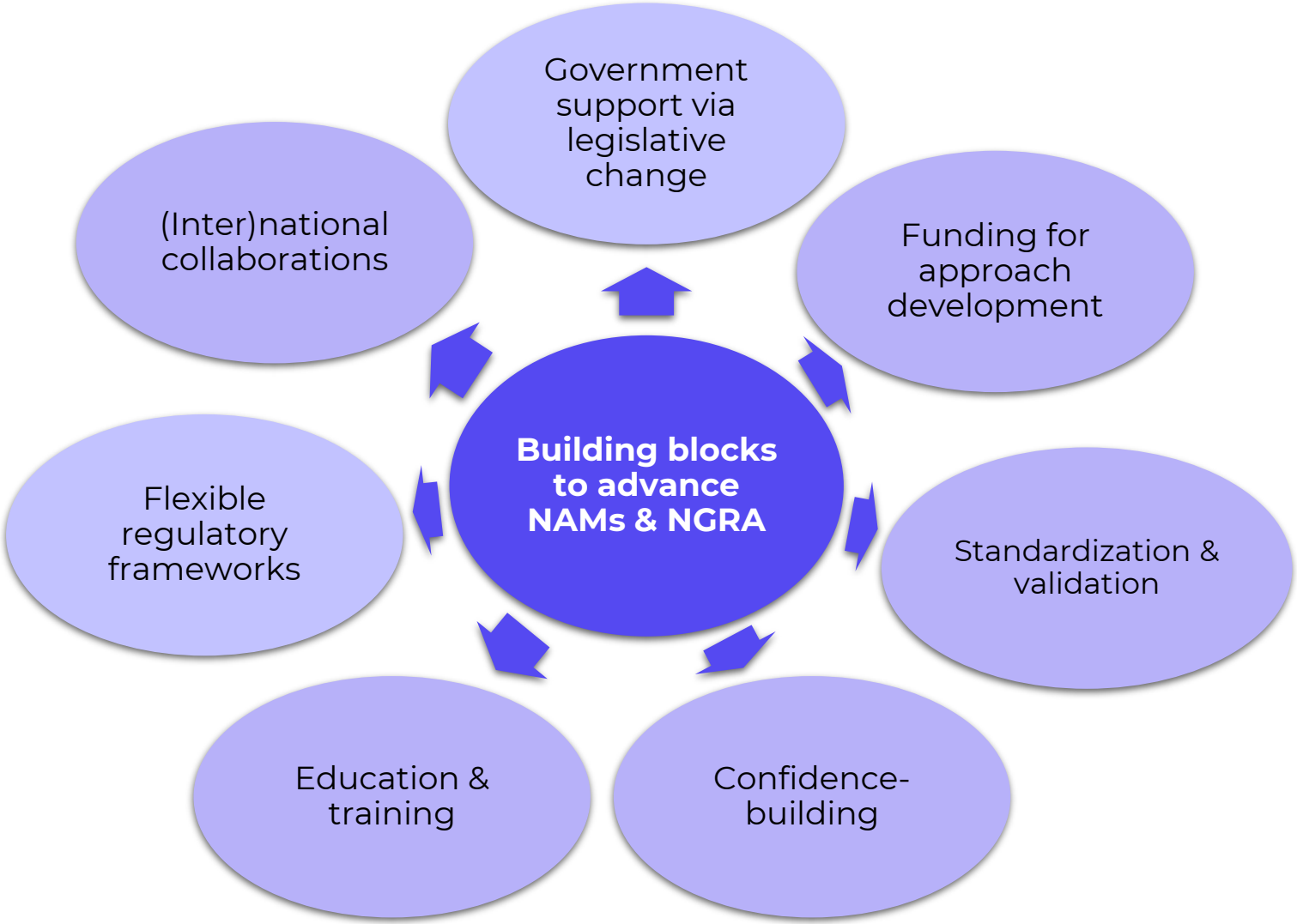


MANY **CASE STUDIES** HAVE BEEN PUBLISHED, OR ARE UNDER DEVELOPMENT, WHICH PROVIDE EXAMPLES OF USING NGRA



ONLY BY **BUILDING CONFIDENCE** IN THE USE OF NAMS AND NGRA FOR REGULATORS, AND FOR END USERS, WILL WE ACHIEVE A PARADIGM SHIFT...

Critical Components For Change



Thank you!

Register for our newsletter

www.iccs-cosmetics.org