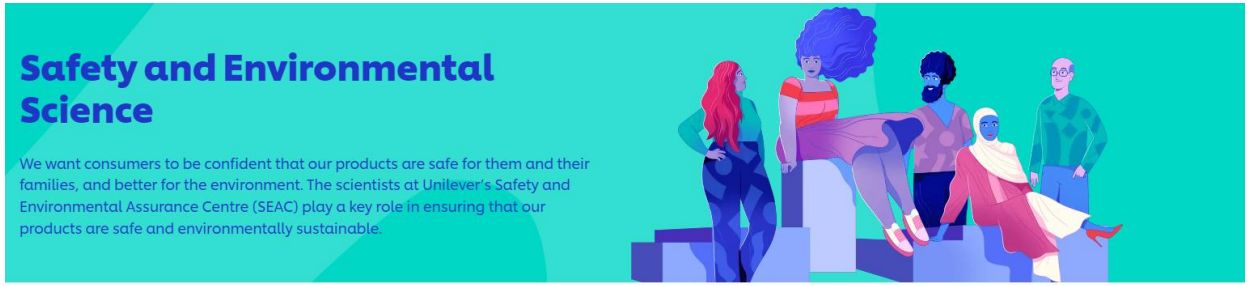
Challenges and opportunities for the use of new approach methods within environmental risk assessment of cosmetic ingredients

Dr Bruno Campos



Leading safety and environmental sustainability sciences

The scientists behind our safe and sustainable products

Safe and sustainable by design

How we build safety and sustainability into every product innovation.

Keeping people and the environment safe

The science-based approaches we use to keep our consumers, workers and the environment safe.

Reducing our environmental impact

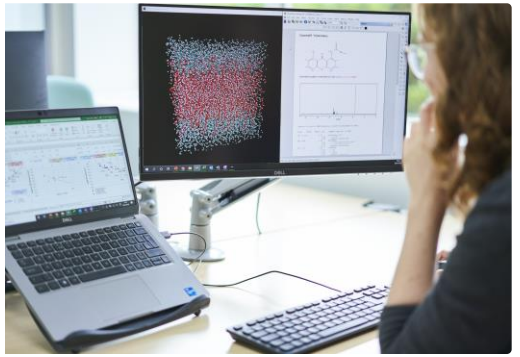
How we harness the latest science to minimise our environmental footprint.

Unilever

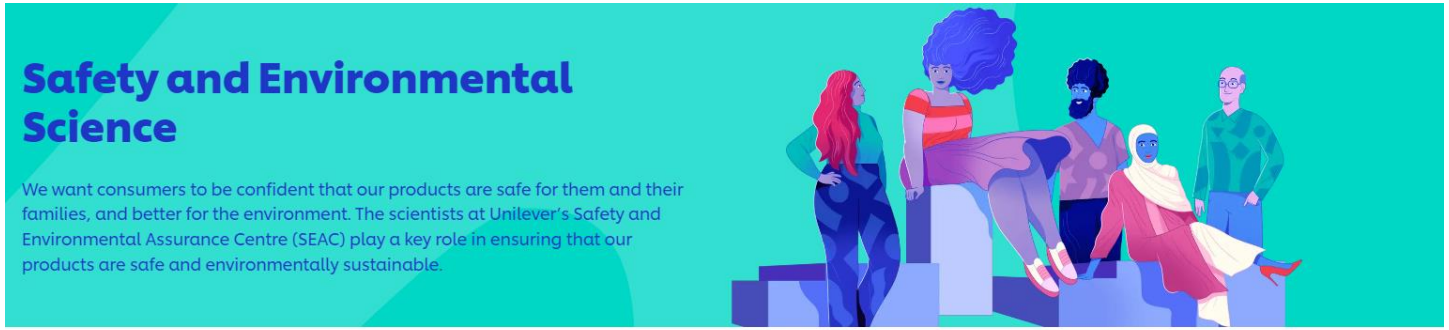
2 Team SEAC's purpose is to protect people & the environment:
Unilever's products & innovations are Safe & Sustainable by Design without animal testing

Safety without Animal Testing:

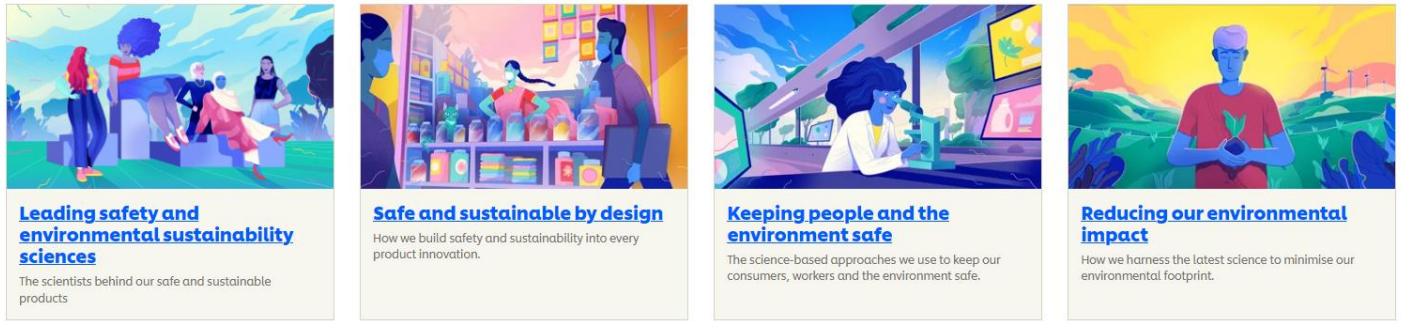
- **Unilever is committed to ending animal testing globally**. We believe in using science, not animals, to assure the safety of our products and their ingredients.



- **Non-animal safety approaches are applied by our leading-edge scientists** in collaboration with world-class researchers & experts.



- We engage with all stakeholders to build shared understanding and promote trust in **our scientific evidence-based approach to decision-making**.



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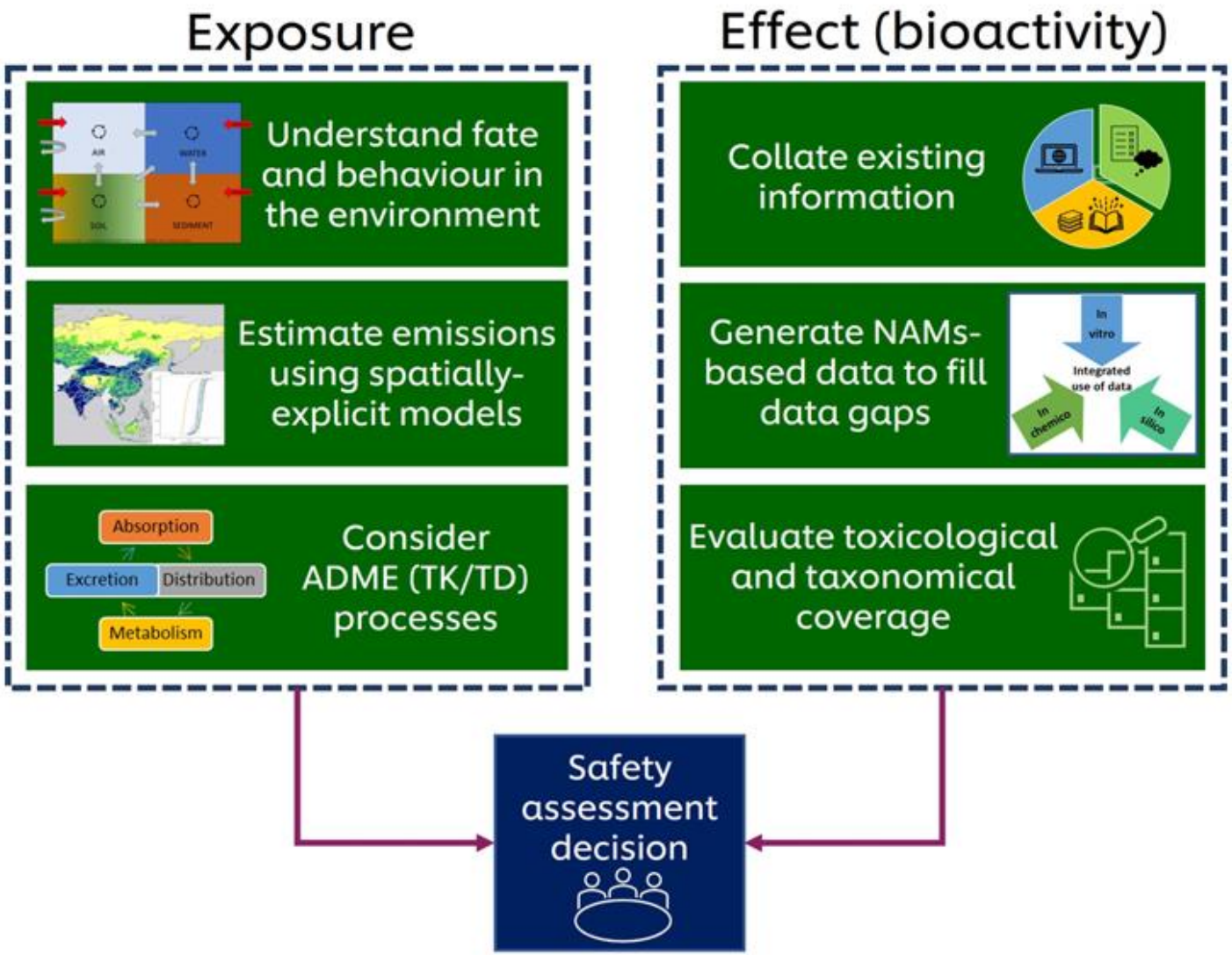
Establishing better environmental protection through Nexgen, mechanistic based environmental risk assessment paradigm shift

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Editorial

Establishing a NexGen, mechanism-based environmental risk assessment paradigm shift: Are we ready yet?

"A concerted effort and orchestration between ALL relevant stakeholders (academia, industry, civil society, regulators, and decision makers) remains the foundation for seamless science translation into fit-for purpose tools for enhanced human and environmental protection"

Rivetti & Campos, IEAM 2023

WHICH TOOL FOR WHICH ENDPOINT?

EXISTING TOOLS AND GAPS

Bioaccumulation	Toxicity to fish	Endocrine disruption
Bioaccumulation in fish: OECD 305	Acute toxicity to juvenile fish: OECD 203	Fish 2 generations: OECD 240
Bioaccumulation in terrestrial oligochetes: OECD 317	Chronic toxicity to fish: OECD 204, 210, 212, 229	Fish sexual development: OECD 229, 230, 234, 240, 148
In vitro clearance trout hepatocytes: OECD 319	Fish cell line acute toxicity: OECD 249	Amphibians: OECD 231, 241
Bioaccumulation in *Halella azteca*: draft test guideline	Fish embryo acute toxicity: OECD 236	Fish embryo estrogen activity (EASZY): OECD 250
TKTD models	In vitro method for chronic toxicity: NONE	Xenopus Eleutheroembryo Thyroid Assay (XETA): OECD 248
		Androgen Disruption Adverse outcome Reporter (Medaka fish) (RADAR): OCDE 251
		Invertebrates: OECD 201, 211, 242, 243 218-219, 222, 220, 225, 226, 232 (Relevance?)
		Effects on vertebrate progeny for cosmetics: NONE

adapted from V. Poulsen 2023

WHICH TOOL FOR WHICH ENDPOINT?

Weight-of-evidence vs 1 on 1 substitution

Fish Acute

- State of the art MoA/ MechoA identification (in-silico profilers, Critical Membrane Burden/ Critical Body Burden/ 'Omics etc.)
  - State of the art grouping/read-across (e.g. omics based grouping)
  - In silico/ QSAR approaches – $logK_{MW}$/$logK_{OW}$ etc.
- In-vitro assays / cell based profiler panels (e.g. RTgill cell (OECD TG 249))
  - PBK/ TK modelling
- Pathway based mechanisms of toxicity across species (X-spp extrapolation/ species sensitivity)
- Exposure thresholds (e.g. EcoTTC)
- Understanding species sensitivity – distributions
- Complementary approaches proposed in the OECD Fish IATA (e.g. Threshold approach)

Fish Chronic

- Approaches as per Fish Acute + the following
  - Acute to Chronic ratios
  - Exposure based waiving – consideration of EcoTTC, biodegradation rates etc.

Fish BCF

- In silico modelling (e.g. BIONIC)
  - Biotransformation measurements - S9/hepatocyte assay (OECD TG 319A/B)
  - Empirical and/or in-silico derivation of relevant partitioning parameters e.g. $logK_{ow}$ and/or $logK_{mw}$
  - Empirical and/or in-silico derivation of pKa
- Non vertebrate bioaccumulation assays (*Hyalella azteca*, RT-gill cell line etc.)
- Exposure based waiving – consideration of removal/biodegradation rates etc and fate in environment

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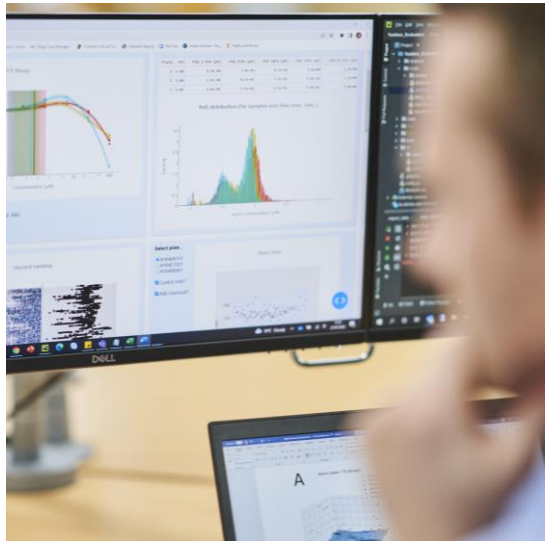
Case study: A proof of concept to demonstrate the applicability of mechanistic info in Environmental safety assessment

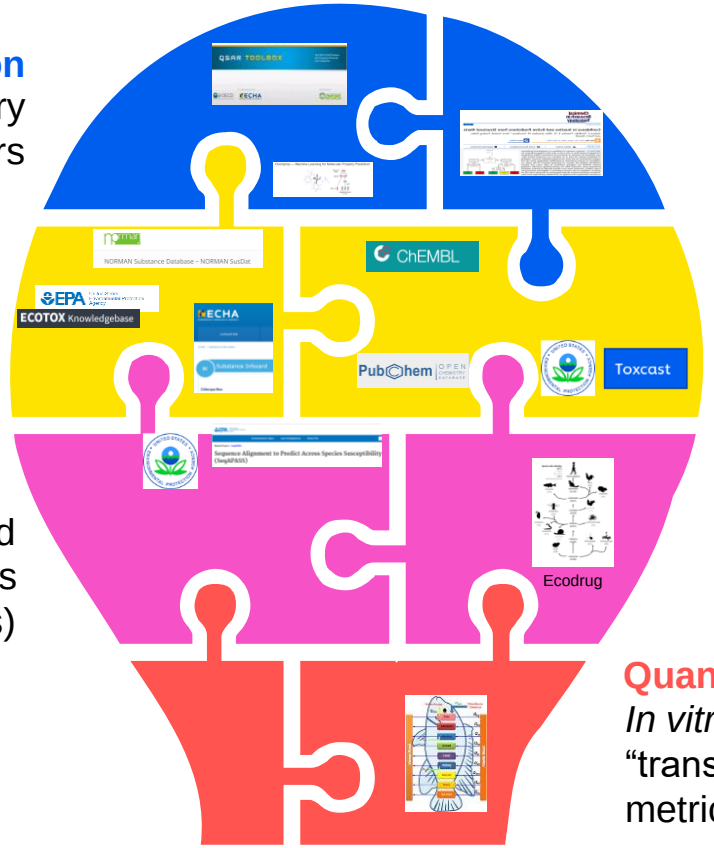


Information gathering process:

Mode of Action identification
Using available scientific and regulatory information and in silico profilers

Species at risk identification
Use of publicly available tools and databases to identify susceptible species (based on targets and processes)

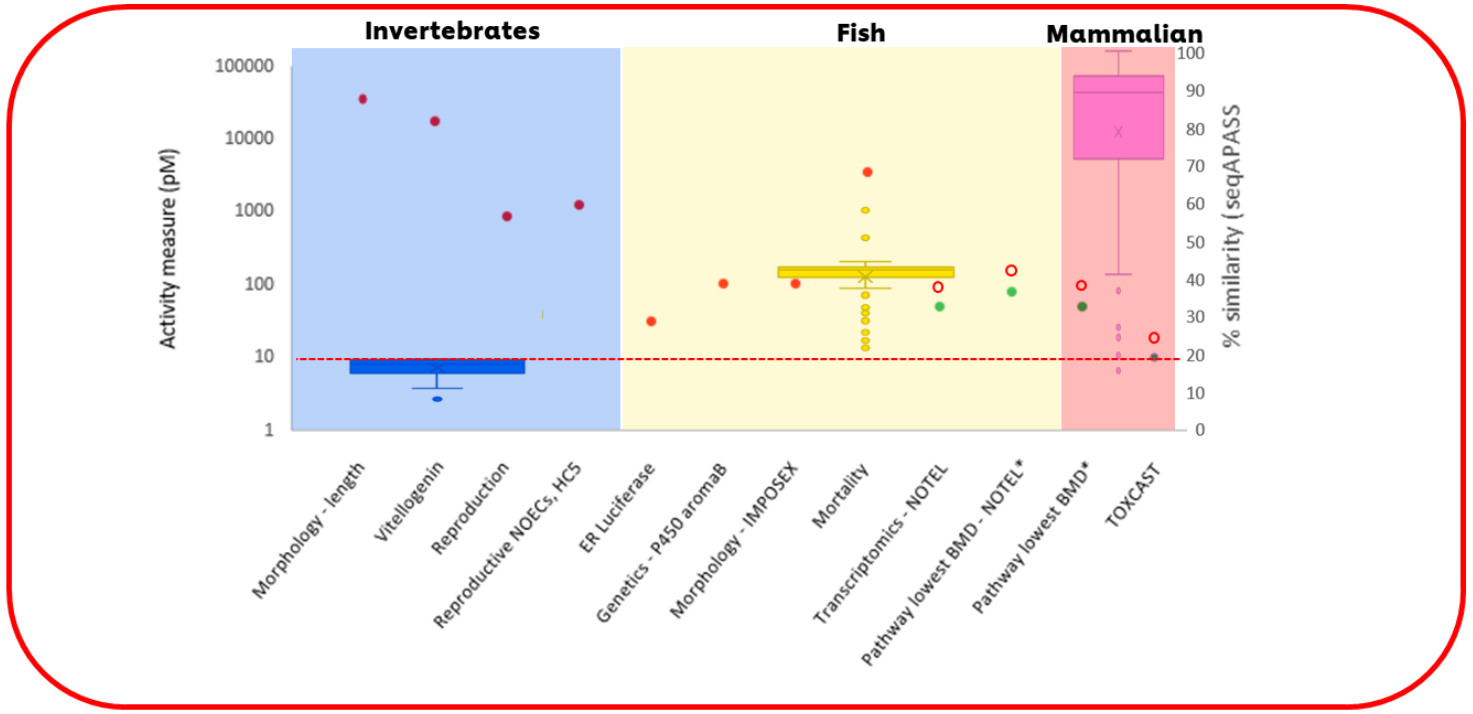
Weight Of Evidence approach
Collate all the information in an intelligible way to guide and support decisions



Hazard Data
Including historical *in vivo* as well as *in vitro* data and *in silico* predictions to generate relevant PoD

Quantitative In Vitro to In Vivo Extrapolation
In vitro and *in vivo* exposures must be “transformed” into comparable exposure metrics requiring robust qIVIVE models

Case-study 1: ethinylestradiol



Microarray analysis


Hepatic gene expression profiling using Genechips in zebrafish exposed to 17α-ethynylestradiol

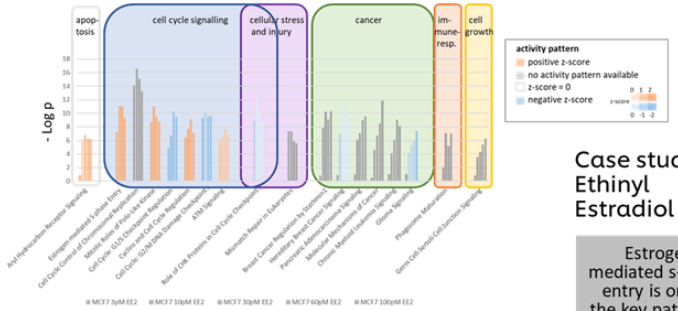
NOTEL 168h* = 50pM

Pathway with lowest BMD at 168h: 78pM

*Threshold FC >2, p < 0.05, a cut of at FDR < 0.1 would change the numbers of DEGs but not the NOTEL

Hoffmann et al., (2006)

Canonical Pathway analysis

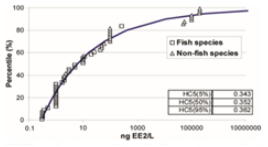


Top 20 pathways predicted by Ingenuity Pathway Analysis (IPA) according to top p-value

Case study: Ethinyl Estradiol (EE2)

Estrogen mediated s-phase entry is one of the key pathways but other pathways are also identified

Literature information



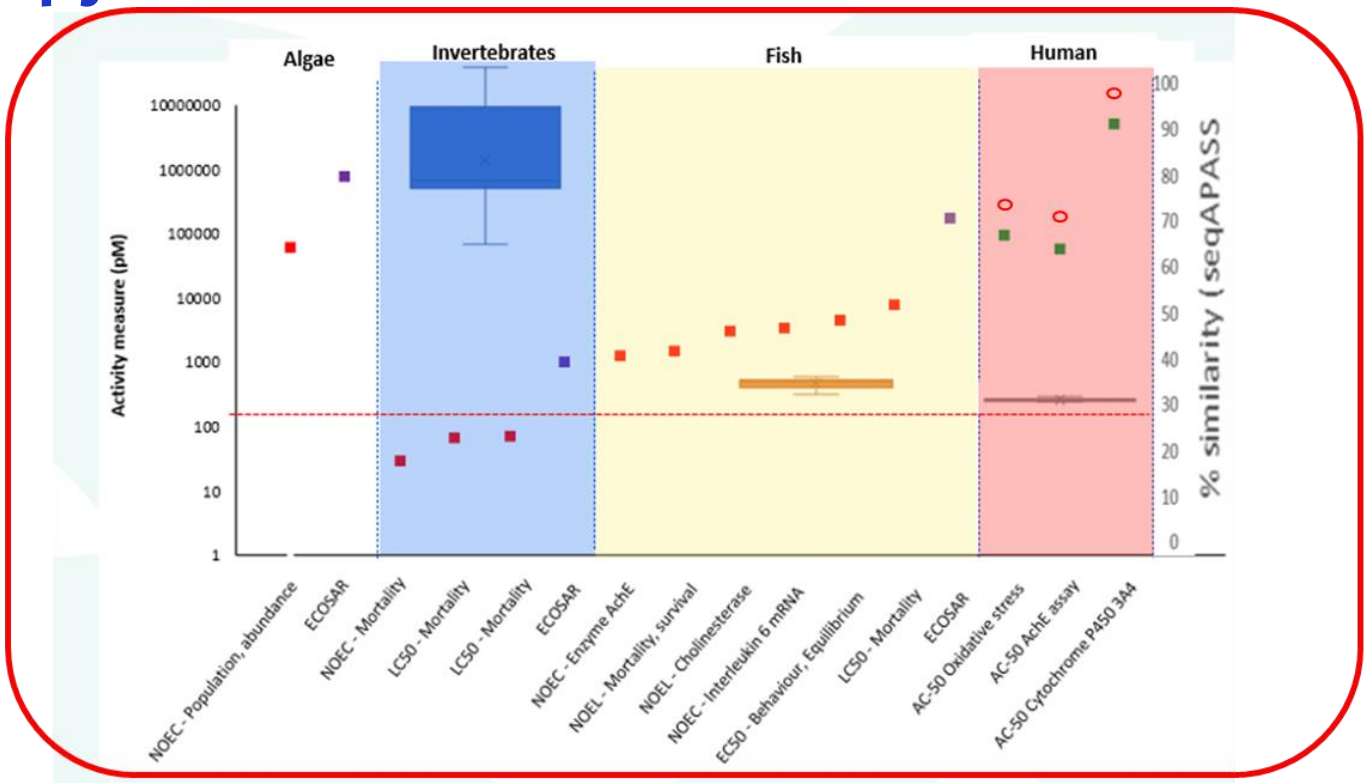
HC5 (50%) = 1200 pM

EC50= 30pM (ER luciferase assay)

Toxcast

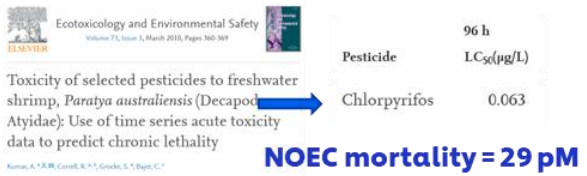
*Note: These data are the property of Unilever Plc and cannot be shared without permission. It has been created for discussion and training purposes only and so may not reflect true experimental values. Unilever does not conduct fish testing including early life stage testing.

Case-study 2: Chlorpyrifos



- *In silico*
- *In vitro*
- *In vivo*
- *In vivo after reverse dosimetry calc*
- *SEQapass act. threshold*

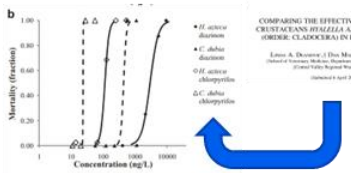
Hazard data


Ecotoxicology and Environmental Safety

Toxicity of selected pesticides to freshwater shrimp, *Paratya australiensis* (Decapod Atyidae): Use of time series acute toxicity data to predict chronic lethality

Pesticide	96 h LC_{50}(µg/L)
Chlorpyrifos	0.063

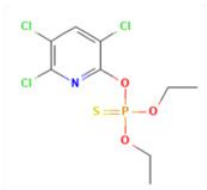
NOEC mortality = 29 pM



COMPARING THE EFFECTIVENESS OF CHRONIC WATER COLUMN TESTS WITH THE CRUSTACEANS *HYALELLA AZTECA* (ORDER: AMPHIPODA) AND *CERIODAPHNIA DUBIA* (ORDER: CLADOCERA) IN DETECTING TOXICITY OF CURRENT-USE INSECTICIDES

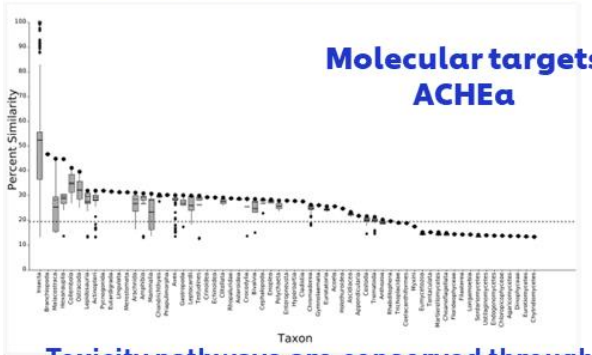
LC50 mortality 66 pM

In vitro data



AchE Assay AC50 = 56.6 nM

Cross-Species Extrapolation analysis



Molecular targets ACHEa

Toxicity pathways are conserved throughout the animal kingdom

*Note: These data are the property of Unilever Plc and cannot be shared without permission. It has been created for discussion and training purposes only and so may not reflect true experimental values. Unilever does not conduct fish testing including early life stage testing.

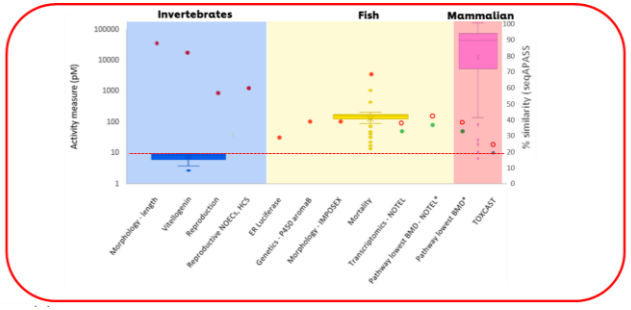
Key highlights

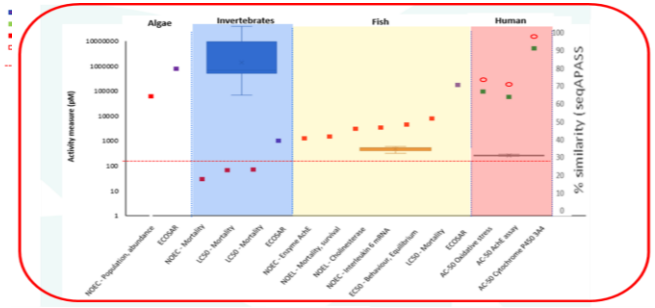
Integration of *in vivo, in vitro and in silico data* in a weight of evidence approach can build confidence in safety decision-making.

- ✓ provides confidence that most sensitive species can be identified (in line with historical knowledge of chemicals);
- ✓ Species sensitivity is in line with MoA and target conservation
- ✓ *in vitro* endpoints seem to be at least as protective as traditional *in vivo*.

Challenges to be addressed

- ➢ Lack of standardised study designs may hinder data usage
- ➢ Challenges for data-poor chemicals
- ➢ No one-size-fit-all approach





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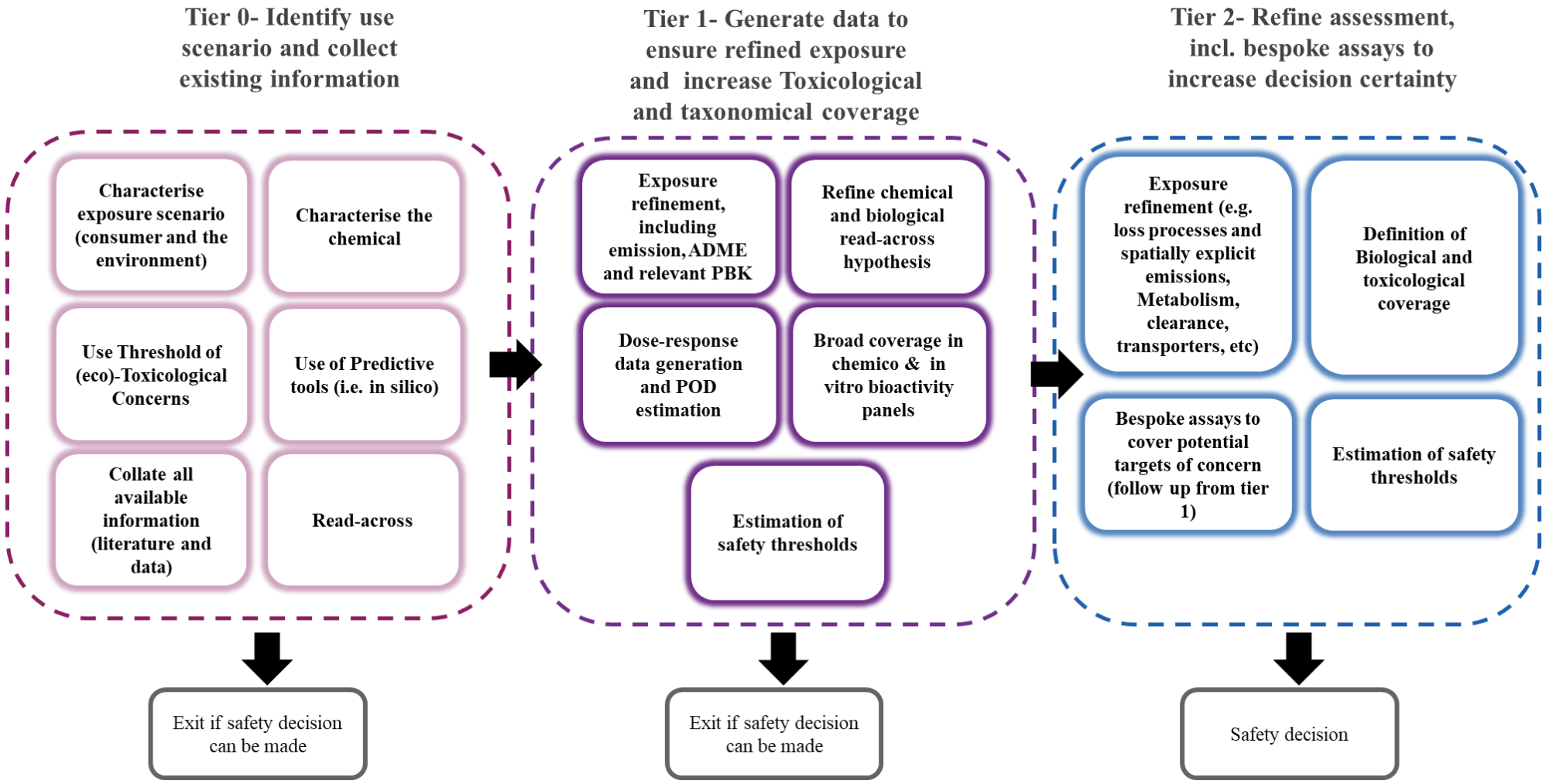
Ultimate goal: Increased integration of human & environmental safety decisions

First step– developing a common framework & language



adapted from *Baltazar et al.* 2020

Take home messages

- Understanding exposure is critical to applying/ interpreting NAMs for safety assessment.
- Tangible opportunities already available to improve environmental protection by applying NAMs approaches and all available information
- Mechanistic understanding allows to move away from black box in vivo studies, to better understand how chemicals impact species and to identify other potential impacts which in vivo studies would not identify.
- There are challenges to address particularly in standardisation and training needs within user communities (Risk Assessors and Regulators)

Unilever

Thank You

“the team”

- Emilia Gattas
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- David Gore
- Yaping Cai
- Katie Endersby
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- Mathura Theiventhran
- Danilo Basili
- Predrag Kukic
- Iris Muller
- Simran Sandhu
- Baile Xu
- and many more...

All underpinned by SEAC science, its scientists and our scientific partners

seac.unilever.com