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Open **2-3 July 2026** Symposium

Maastricht
The Netherlands

ASPA: an NGRA workflow for NAM-based safety assessments

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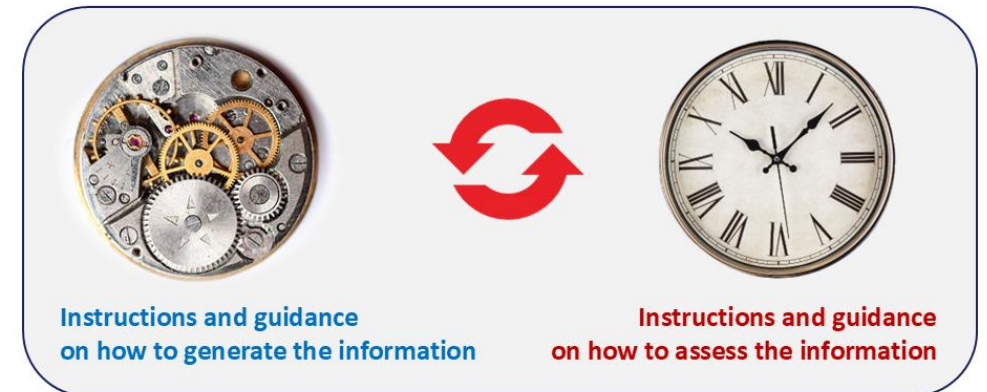
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Project: RISK-HUNT3R

aspis-cluster.eu



- The ASPIS cluster aims to **operationalize** NGRA, by developing a **well-guided NGRA workflow** for safety assessment of chemicals
- This workflow serves as **guidance** on **data generation** and **data interpretation**:
 - Defines a tiered approach on what NAMs to use for each step
 - At which steps to obtain, integrate and evaluate data (incl. uncertainty assessment)
 - How to put data into a context of a hazard or risk assessment scenario

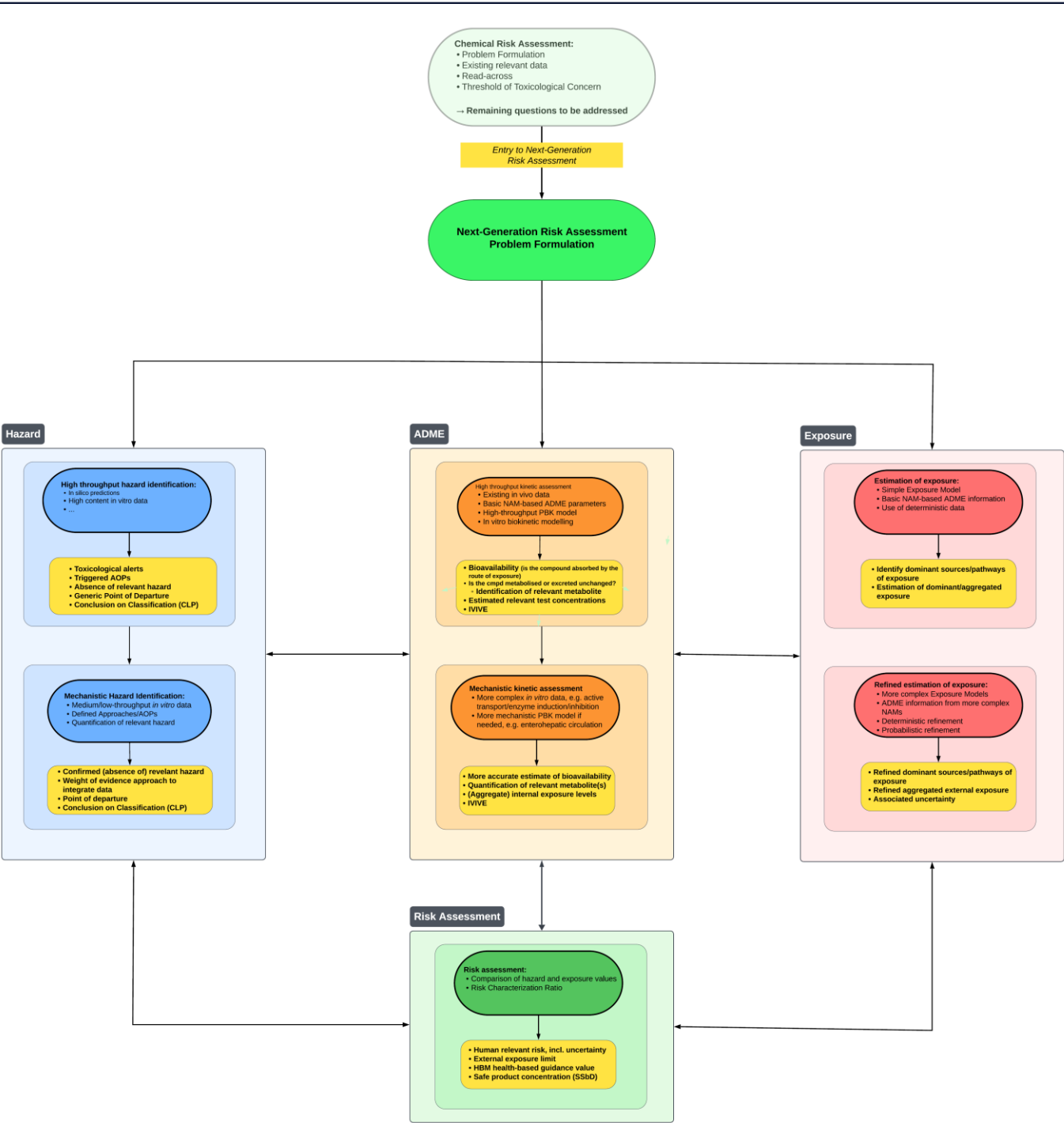


Three NAM-based assessment pillars

- **Hazard**
- **ADME**
- **Exposure**

Each pillar follows a tiered approach.

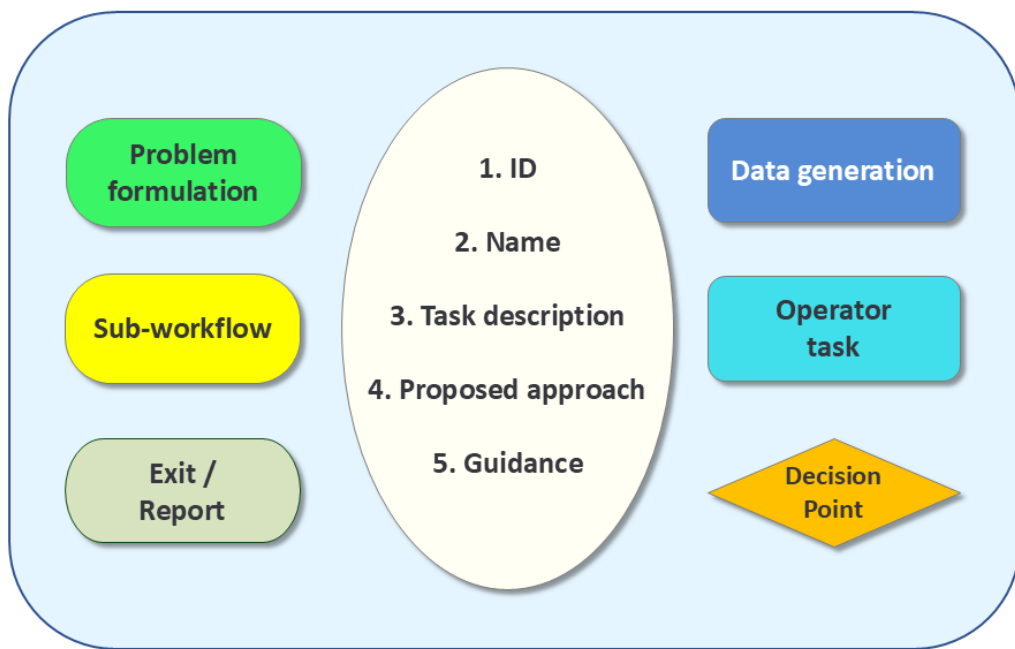
Together, they allow for a **hazard or risk assessment**.



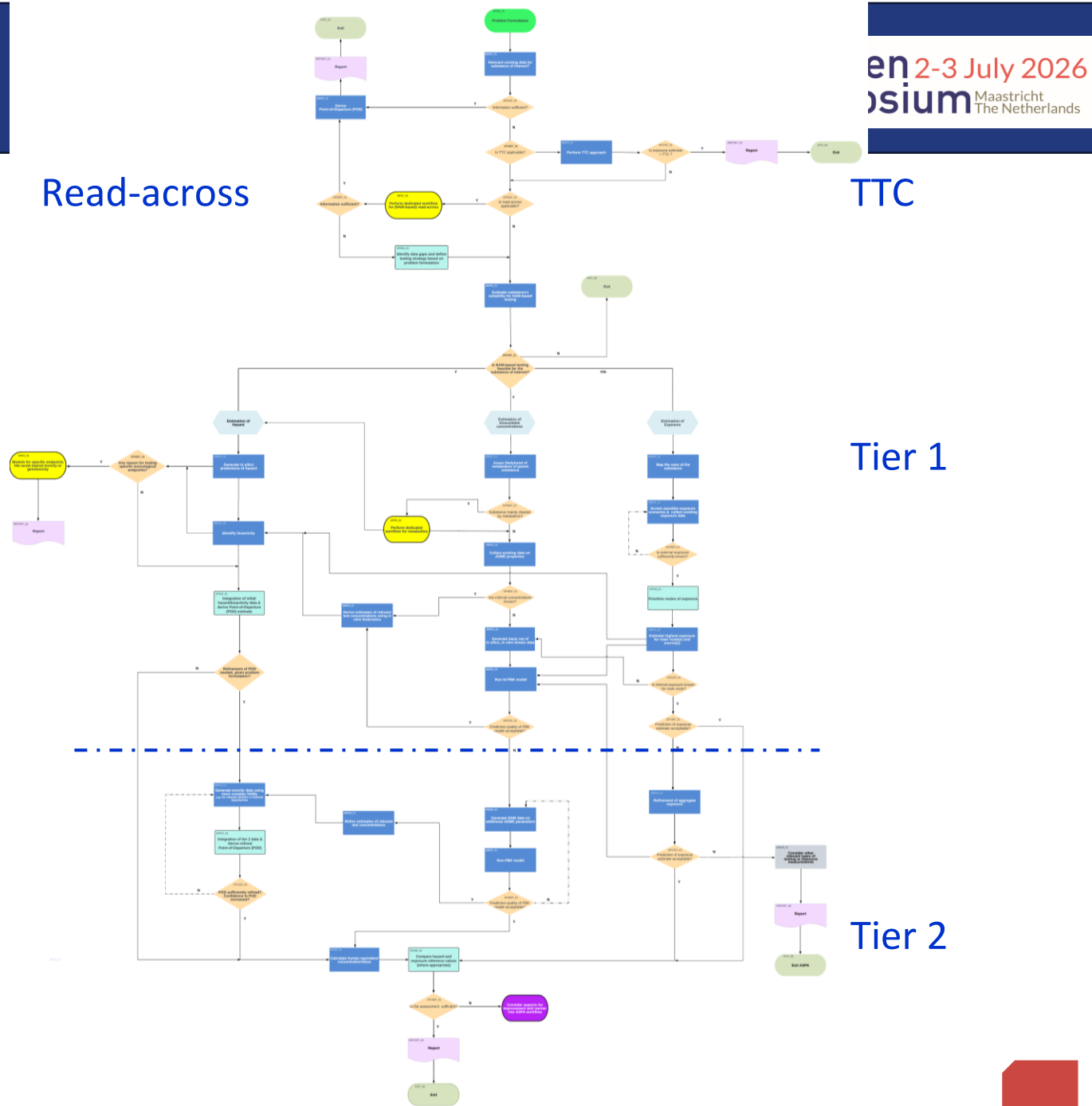


ASP v3.0

- >50 building blocks
- Clearly defined modular structure
 - Problem formulation
 - Data generation task
 - Operator tasks (data analysis)
 - Decision points
 - Exit to risk assessment



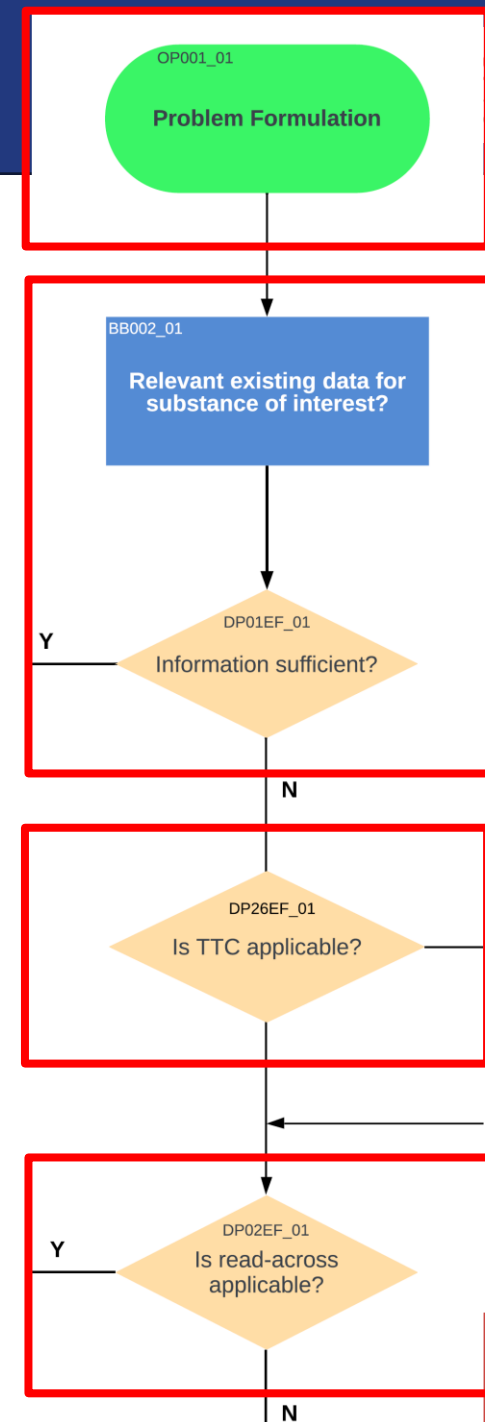
Read-across

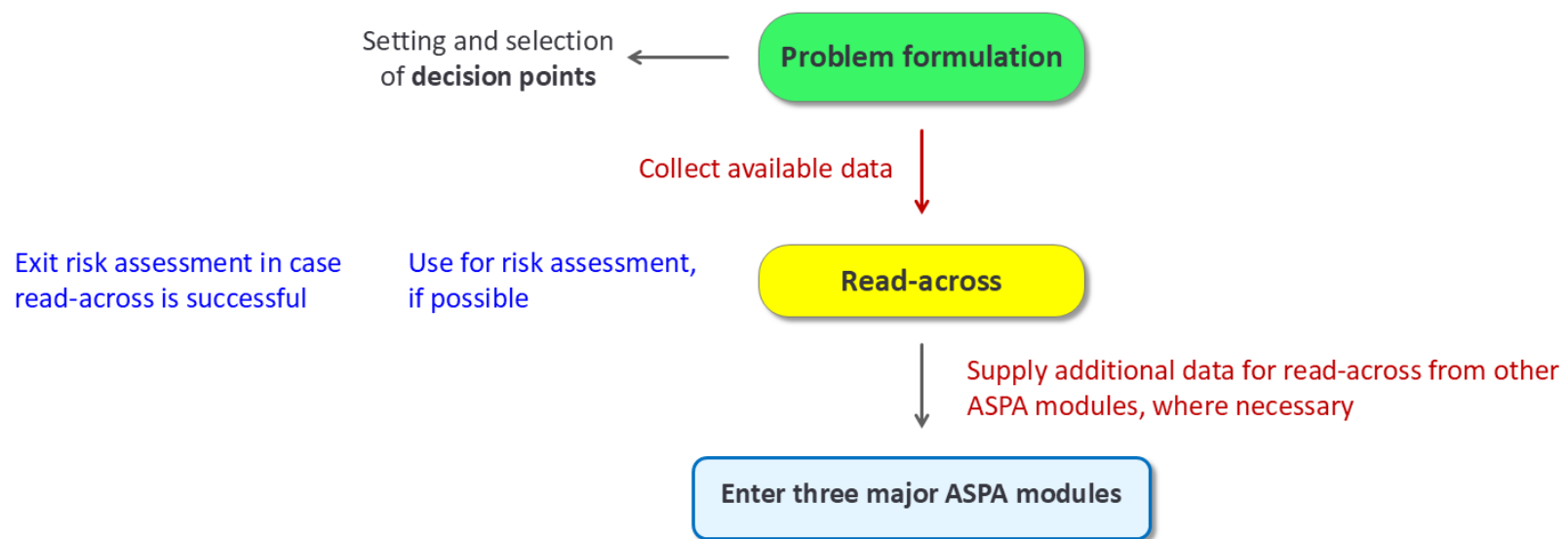




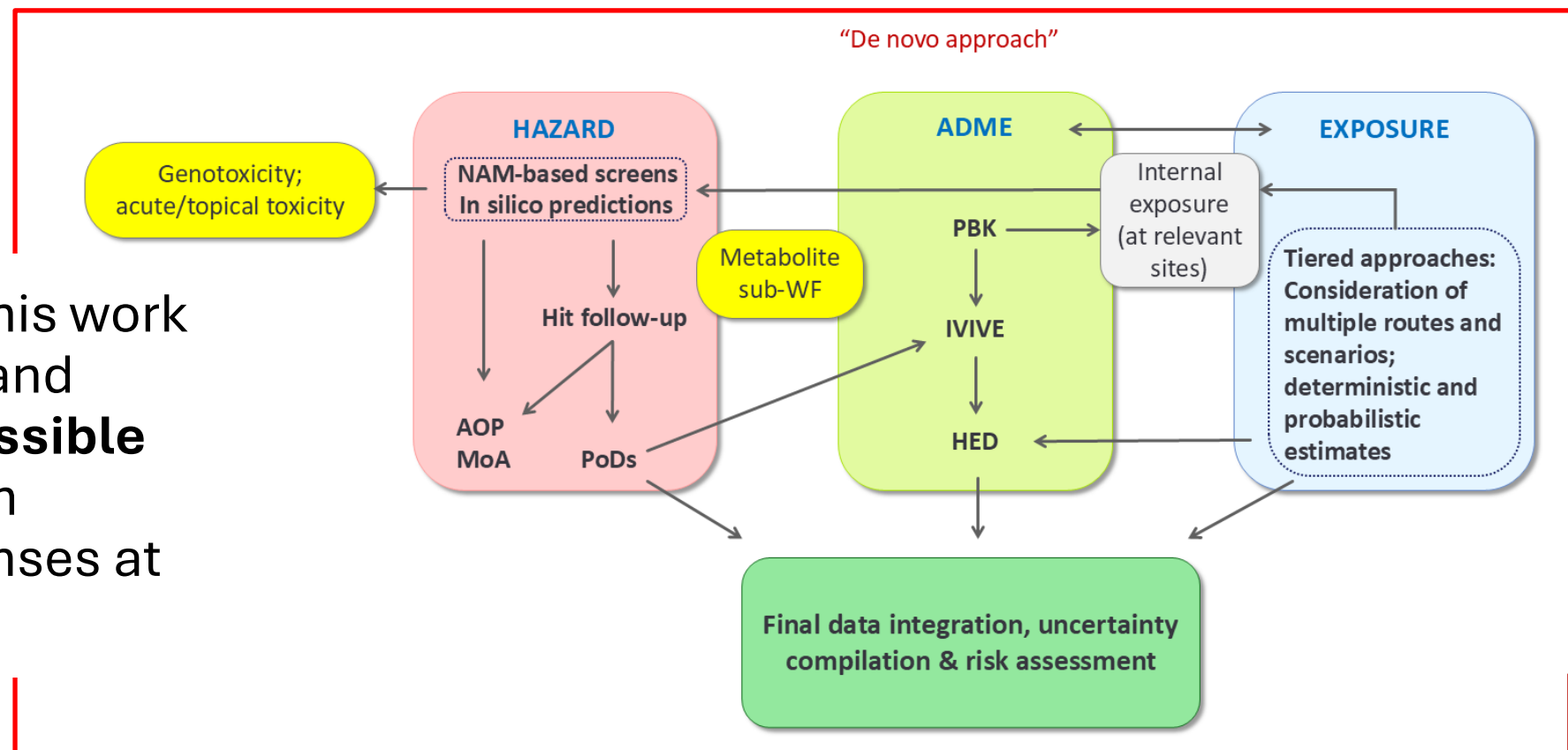
ASPA: generic part

- ASPA starts with the problem formulation: what (regulatory) question do you aim to address?
 - Hazard or risk assessment?
 - Specific population?
 - Context of use? Specific regulation applicable?
 - Resources/time available?
- First, other options are explored:
 - Are **existing data** available that are informative and of sufficient quality?
 - Is the **TTC (Threshold of Toxicological Concern)** applicable?
 - Is **read-across** an option to address the problem?





! In practice, some of this work may occur in parallel, and **different flows are possible** (dependent on problem formulation and responses at decision points)





- Online software which formalizes the reporting process in a reproducible and standardized fashion
- Guides an operator step-by-step through the ASPA workflow
- Generates a complete and comprehensive report
- Report includes all applied methods, operator activities and intermediate decisions
- Practical examples illustrating the broader applicability of ASPA across various regulations and problem formulations are provided through case studies



PTBBA

PTBBA (NUR87V99A2)

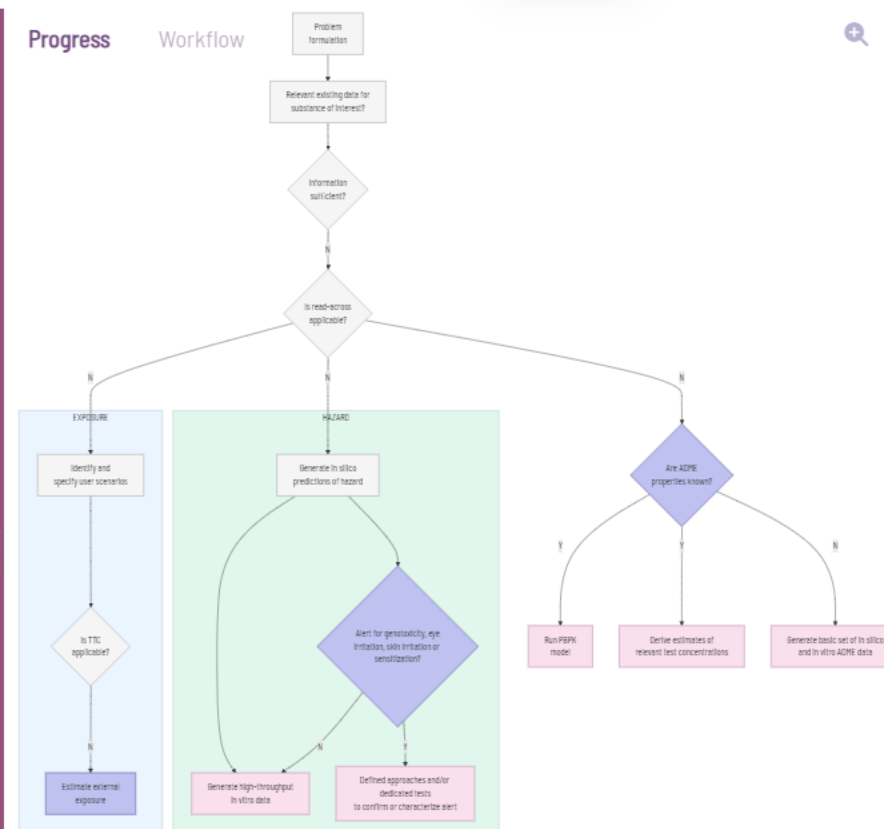
4-tert-butylbenzoic acid (PTBBA) case study

Substances	Name	CAS-RN	Characteristics	Structure
	4-tert-butylbenzoic acid 98-73-7			
Endpoint	Systemic repeated dose toxicity			
AR				
Species	Human			
Framework	ECHA			
Summary	Risk assessment of industrial chemical using NAMs			
Status	Workflow	workflow.csv		
	Step	8		
	Active nodes	BB009_01	Generate high-throughput in vitro data	
		DP03EF_01	Are ADME properties known?	
Read Users		DP06EF_01	Alert for genotoxicity, eye irritation, skin irritation or sensitization?	
		BB023_01	Estimate external exposure	
Write Users				

Progress

Workflow

Reset



Overview

General information

Propylparaben

RA progress

10 Tasks completed

11 Steps

0 Notes

Download:

Generate

Attachments

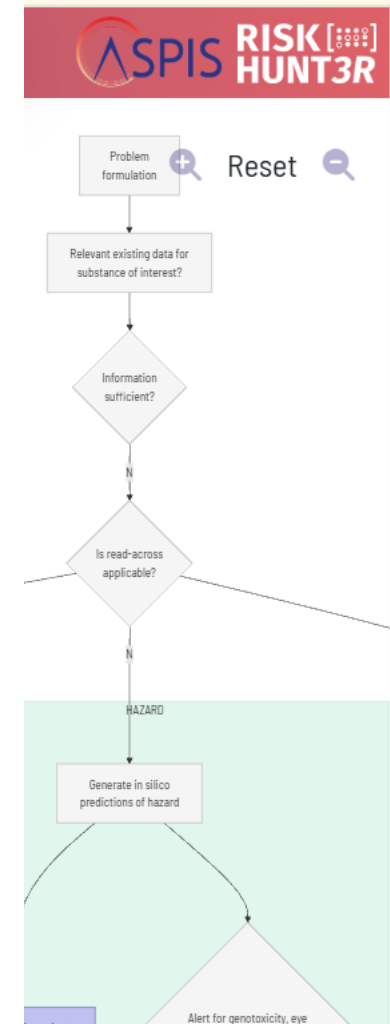
Text

Propylparaben case study - consumer exposure scenario

Report date 2025-09-03

Table of Contents

Table of Contents	1
1. General information.....	2
2. Preliminary.....	2
2.1 Problem formulation (BB001_01).....	2
2.2 Relevant existing data for substance of interest? (BB002_01).....	3
2.3 Information sufficient? (DP01EF_01)	4
2.4 Is read-across applicable? (DP02EF_01)	4
3. Hazard	5
3.1 Generate in silico predictions of hazard (BB008_01).....	5
3.2 Alert for genotoxicity, eye irritation, skin irritation or sensitization? (DP06EF_01).....	6
3.3 Defined approaches and/or dedicated tests to confirm or characterize alert (BB011_01)	6
4. ADME	7





ASPA handbook

General

Hazard

ADME

Exposure

Integration

Problem formulation (BB001_01)



Description

Proposed approach

Guidance

Connections

Relevant existing data for
substance of interest? (BB002_01)

Information sufficient? (DP01EF_01)

Derive Point-of-Departure POD
(BB003_01)

Is read-across applicable?
(DP02EF_01)

Perform dedicated workflow for
NAM-based read-across (WF01_01)

Information sufficient? (DP03EF_01)

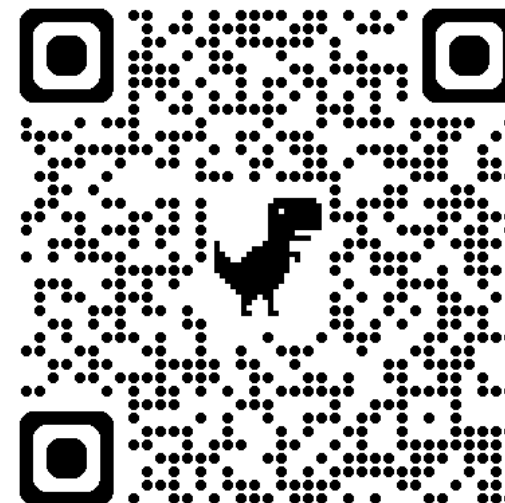
Identify data gap and testing
strategy based on problem
formulation (BB040_01)

Is NAM -based testing feasible for
the substance of interest?
(DP04EF_01)



Enter a description of the problem formulation, which is aimed at defining the purpose and scope of a risk assessment and the depth of the analysis. Please include the relevant regulation(s) that must be considered if an assessment is required for regulatory purposes. Also include the envisaged exposure scenario(s), the substance definition, etc. Give an indication on the level/uncertainty limits of the desired information. If possible: specify the areas of toxicity (knowledge gap to be filled), the level of need for mechanistic understanding and potential exit points (sufficient information reached)

Can be found on
<https://namastox.upf.edu/handbook>



What does it do?

Guide the user through the workflow based on a compound specific problem formulation

Reports all methods, analysed data and decisions

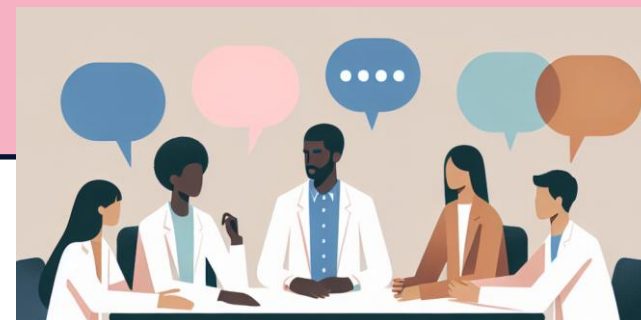
Provides decision criteria, which can be applied by the user (e.g. metabolite work flow)

What does it not do?

It is not a „one click NGRA prediction tool“

The user has to provide the data and assessments – thinking is ~~allowed~~ mandatory

The user has to take decisions





- Building **trust and confidence** in and enhancing **understanding** of NAMs is a key requirement for the implementation of NAMs, to achieve **shift in mindset**
 - Moving towards next-generation risk assessment involves **changes** in the type of data and info to be assessed, and **changes** in type (magnitude?) of the associated uncertainties
- **Case studies** are needed to demonstrate where and how NAMs can be used for regulatory decision-making





- **Context:** CLP (Classification, Labelling and Packaging), *i.e.*, hazard identification, with focus on carcinogenicity
- **Proof-of-concept:** focus on single MOA
 - Activation of peroxisome proliferation activated receptor α (PPAR α)
 - Well-known mode of action (MOA) for liver tumor formation in rodents

PPAR α activation → increase in enzyme activity → increase in cell proliferation
→ increase in hepatocellular tumors



Key question:

Can *in silico* and *in vitro* NAMs provide relevant mechanistic information on carcinogenic potential, particularly for chemicals acting through PPAR α -mediated pathways?

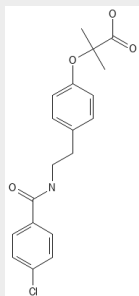
Assumption is that existing data are insufficient or not available



Carcinogenic substances

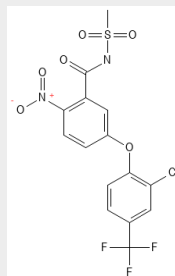
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PPAR α activation



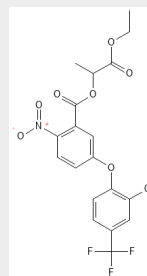
Bezafibrate

Liver; rats/mice



Fomesafen

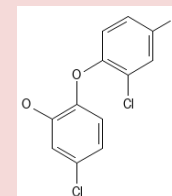
Liver; mice



Lactofen

Liver & stomach; mice

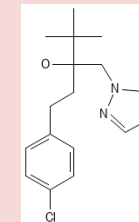
PPAR α + CAR activation



Triclosan

Liver tumor; mice

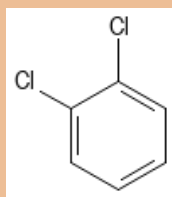
PPAR α activation + other activities



Tebuconazole

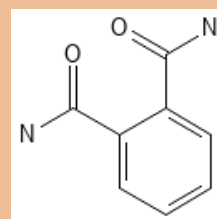
Liver tumor; mice

Non-carcinogenic substances



1,2-Dichlorobenzene

None



Phthalamide

None

Carcinogenicity (or lack thereof) was assessed in studies performed according to OECD TG 451/453

All carcinogenic substances target the liver



Hazard

- *In silico*: physicochemical parameters, QSARs (OECD QSAR Toolbox, Danish (Q)SAR Database)
- *In vitro*: CALUX, Cell Painting Plus, ToxProfiler, transcriptomics and metabolomics in primary human hepatocytes, HDAC inhibitor

Toxicokinetics

- ADMET predictor
- PBK modelling

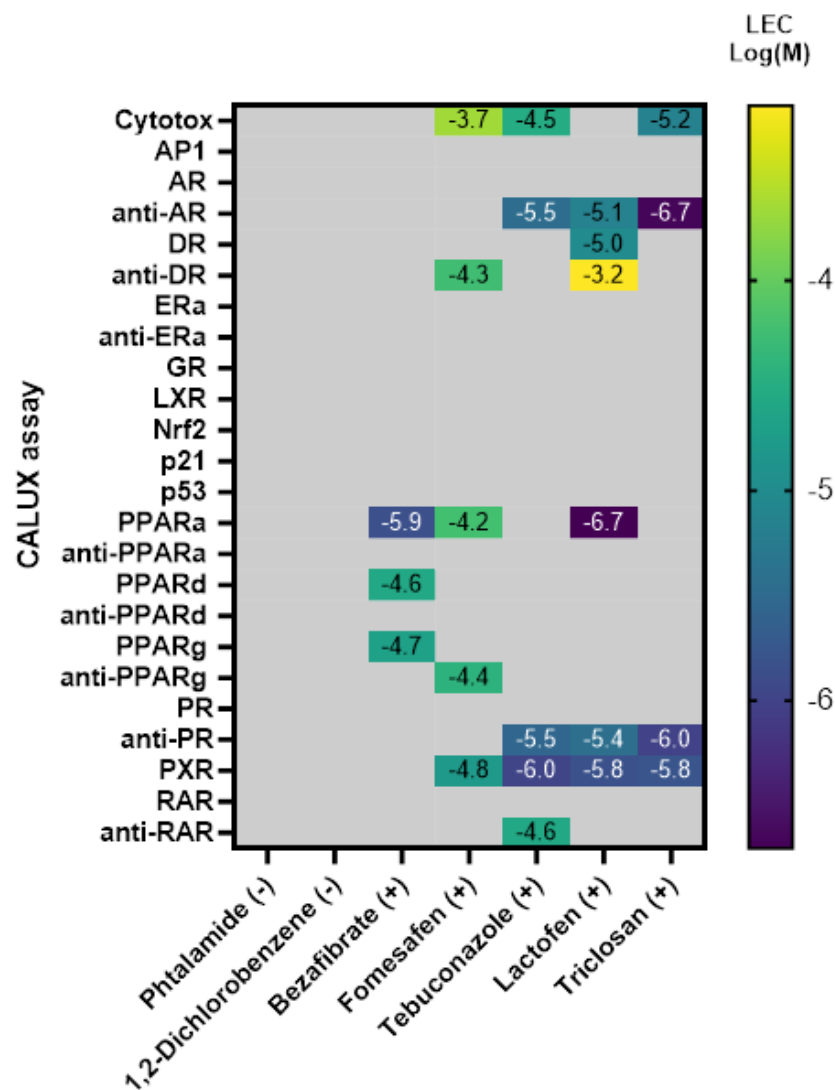
Should be considered as a case study for **tier 1** in ASPA



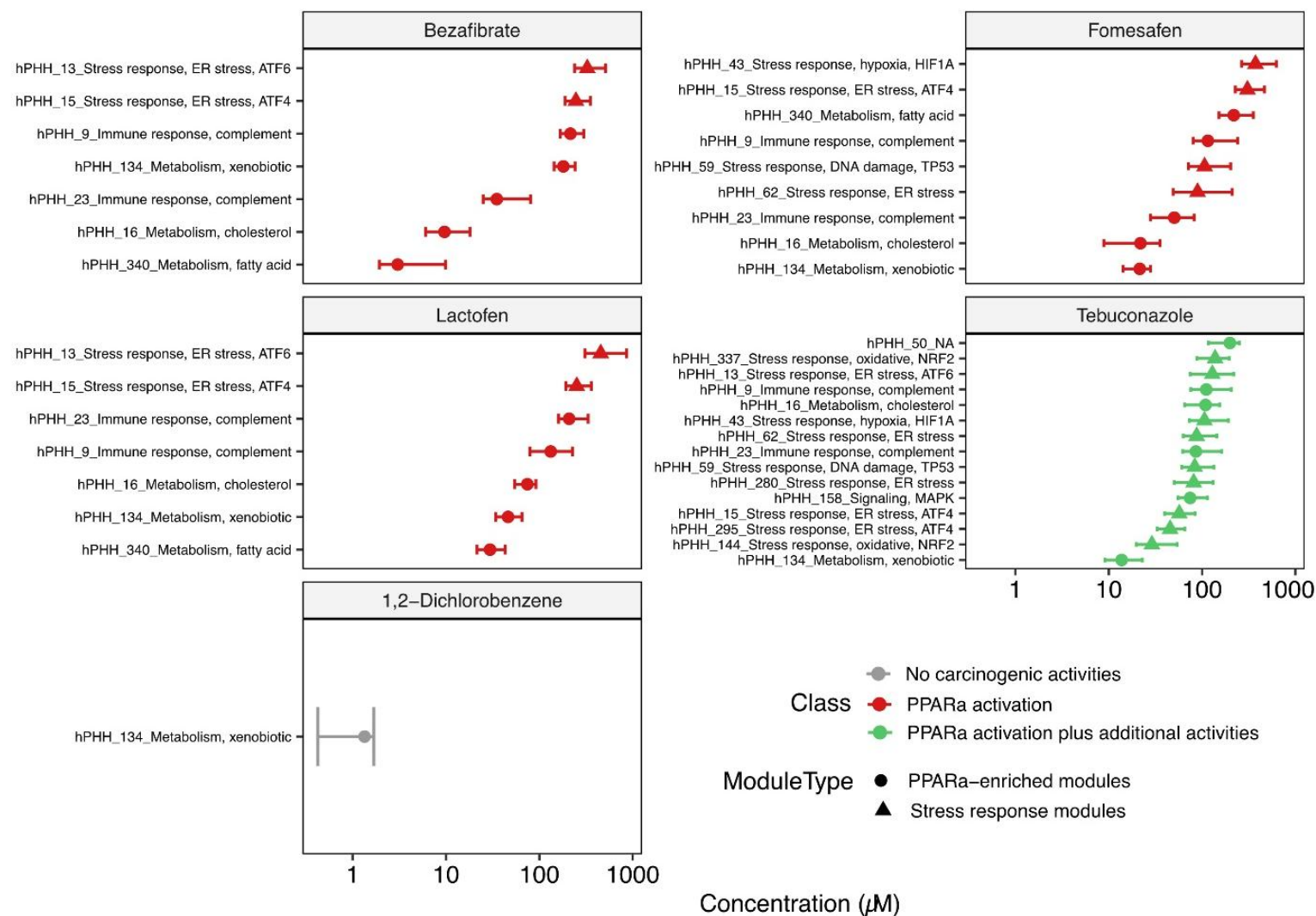
NAM Results: examples

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CALUX



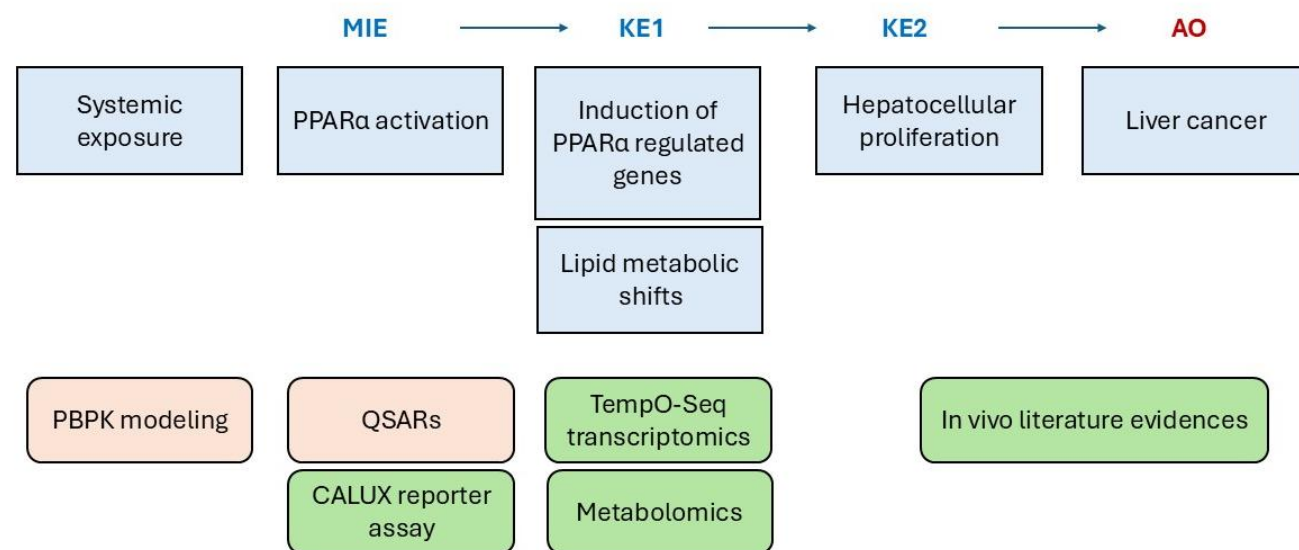
Transcriptomics in PHH





Weight of Evidence approach

- Assessment of strength of evidence from different NAMs:
 - Guided by AOP
 - Based on NAM results obtained
- Assessment of carcinogenic potential for seven substances:
 - Evaluation of NAM data
 - Combining NAM data with evidence for preneoplastic lesions from sub-chronic repeated dose toxicity studies





- NAM-based approach for **Classification, Labelling and Packaging (CLP)**, focused on Developmental neurotoxicity (DNT; as part of reproductive toxicity category)
- NAM-based **risk assessment** of industrial chemical
- NAM-based **hazard characterization**, focused on DNT
- **Prioritization** of chemicals of higher level of concern: using NAMs to discriminate between high toxic *versus* low toxic substances
- NAM-based approach for **read-across**, applied to selected set of conazoles
- NAM-based **risk assessment** of propylparaben
- NAM-based approach for **CLP** assessment, focused on carcinogenicity
- *In vitro* **transcriptomics** for mechanism-based carcinogenicity assessment

Submitted to and discussed as **OECD IATA case studies**



Highly valuable discussions because of **technical** and **societal** aspects



ASPIS has made a significant contribution to the operationalization of NGRA!

Next, we need to:

- Perform **NGRA case studies** covering different industry sectors and different regulations
- Discuss outcomes with wide group of **stakeholders** (incl. end-users): what works well and what does not work good enough (yet)?
- **Raise awareness, prioritize** the development of specific **AOPs** and **NAMs**, **build trust** and **modify regulations** where needed → may require **transition** period



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**Together, we'll make
NGRA happen!**



**THANK
YOU**