ASPIS

Open Symposium 2-3 July 2026 Maastricht The Netherlands

ASPA: an NGRA workflow for NAM-based safety assessments

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ASPA: workflow for NGRA

- 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

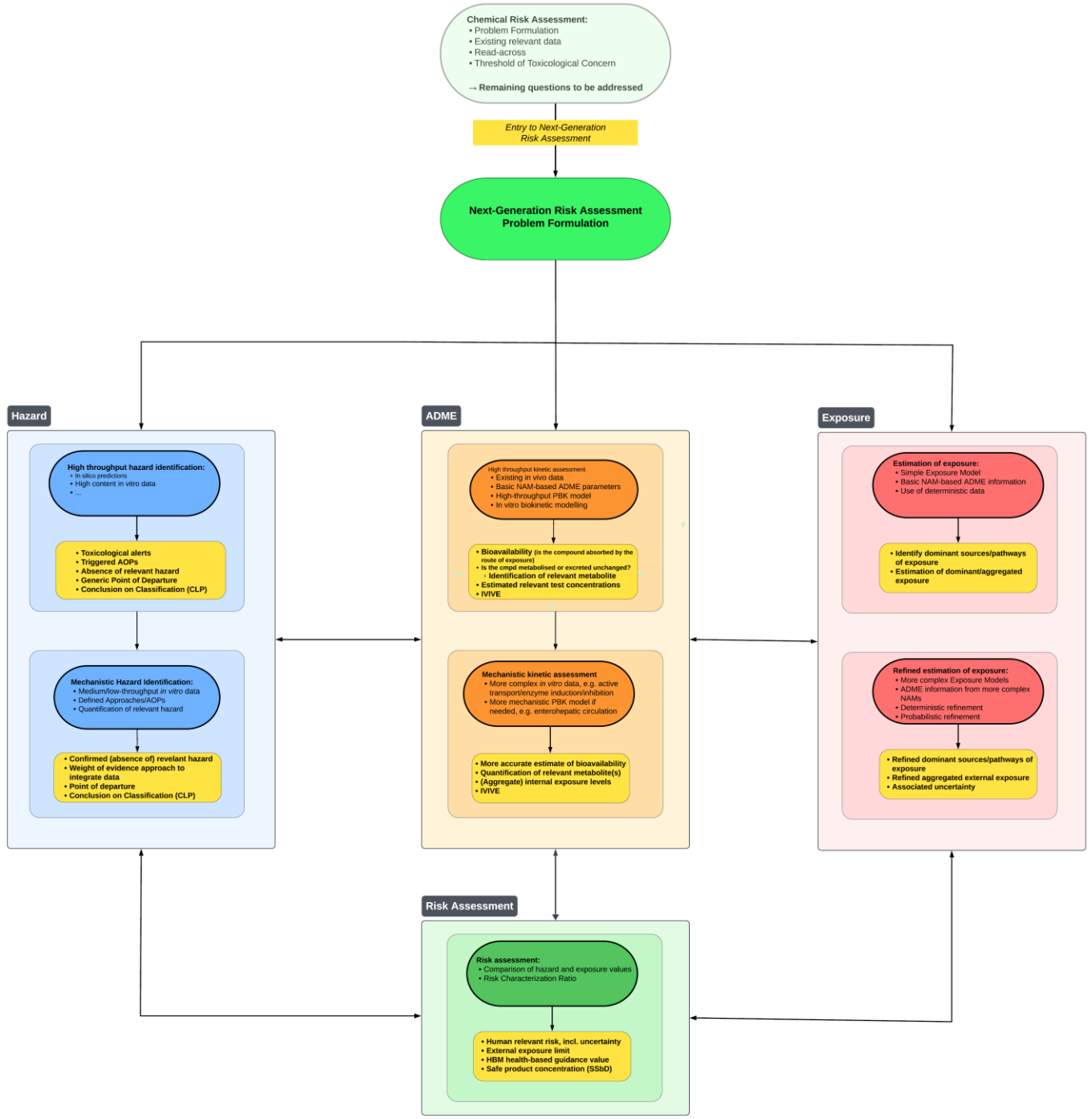
High level view of ASPA

Three NAM-based assessment pillars

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

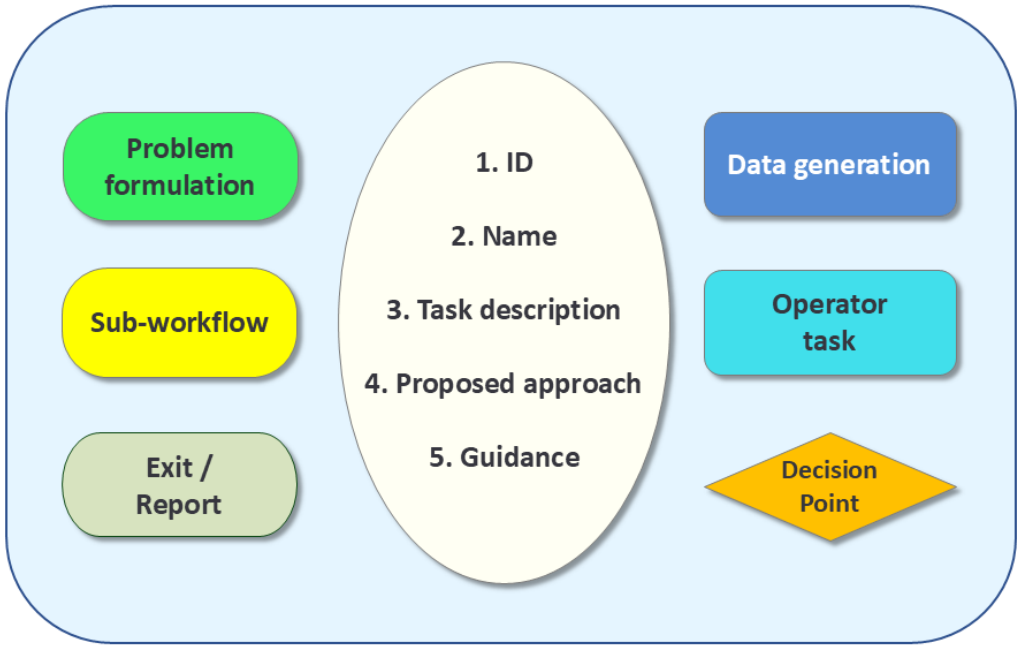
Each pillar follows a tiered approach.

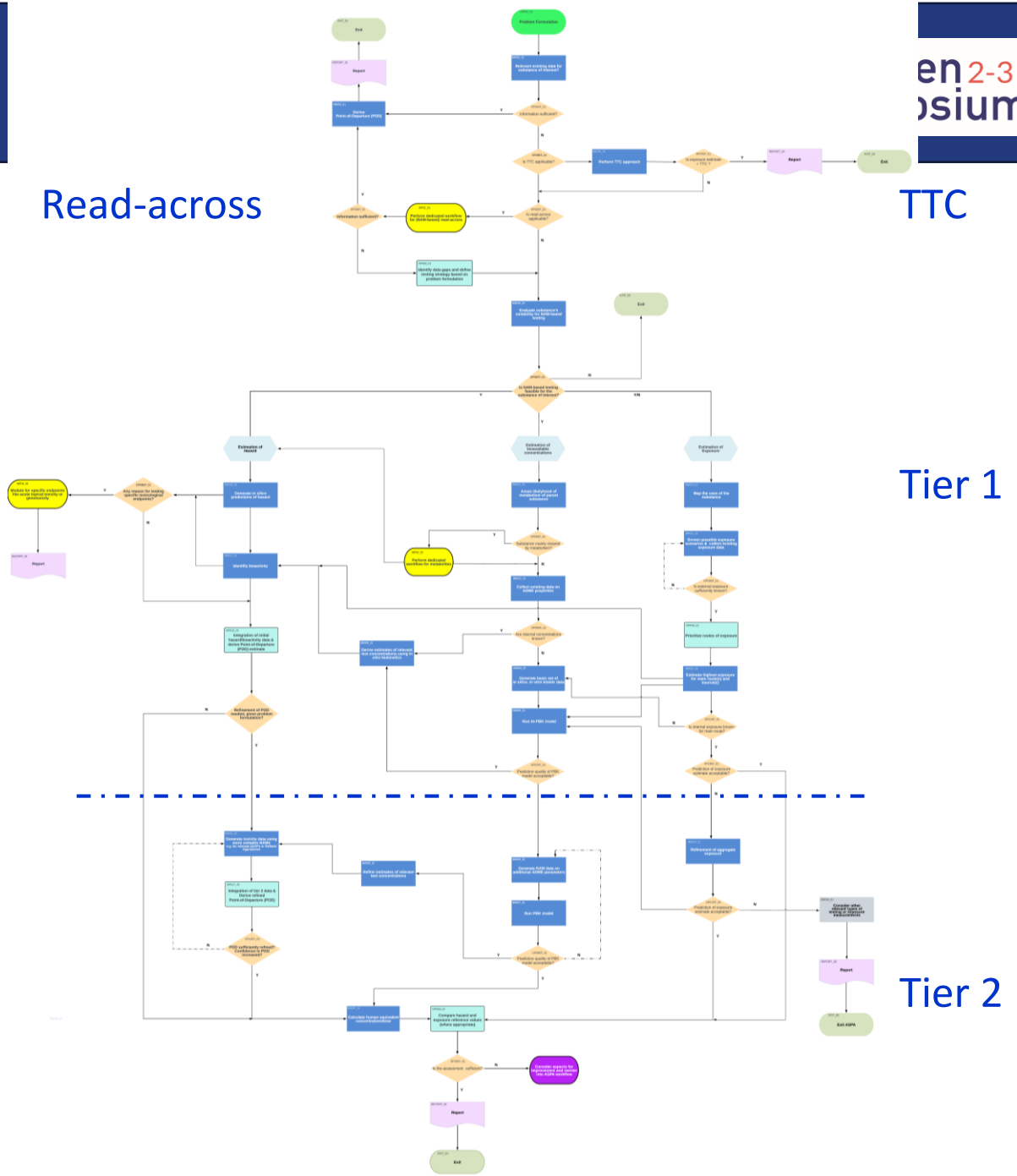
Together, they allow for a **hazard** or **risk assessment.**



ASPA v3.0

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

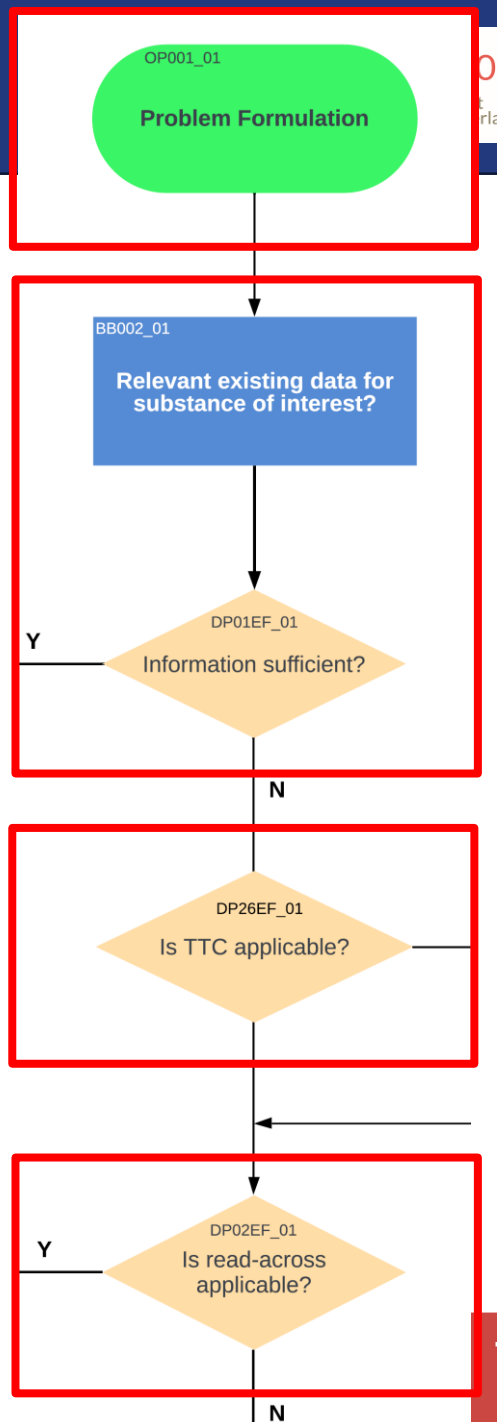




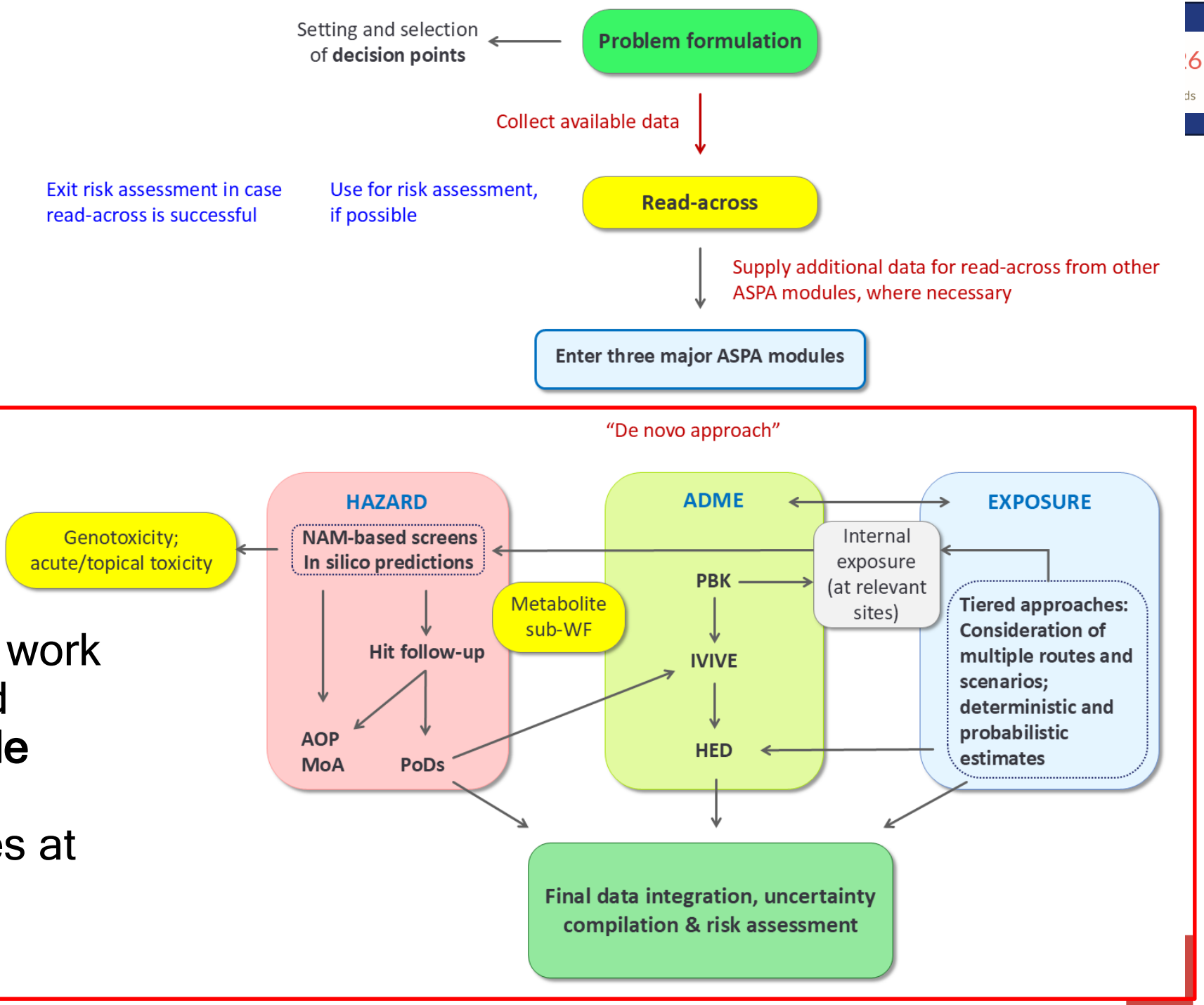
ASPA: generic part

- 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?



ASPA Overview



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

ASPA-*assist*

- 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

ASPA-*assist*

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Overview | General information | Tasks | Decisions | Notes | Report | Help | About | mirjam | ASPIS RISK HUNT3R

PTBBA

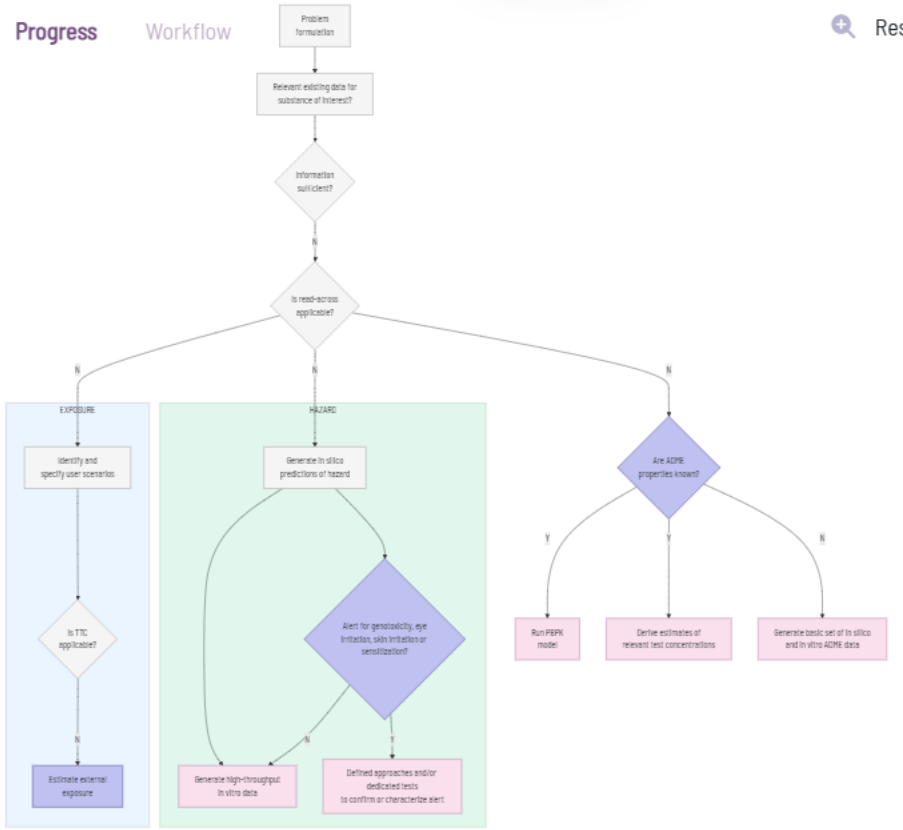
PTBBA (NUR87V99A2)

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?
		DP06EF_01	Alert for genotoxicity, eye irritation, skin irritation or sensitization?
		BB023_01	Estimate external exposure
Read Users	*		
Write Users	*		

Progress | Workflow | Reset



ASPA-*assist* ensures transparent reporting

Overview | General informati

Propylparaben

RA progress

10 Tasks completed

11 Steps

0 Notes

Download:

Attachments

Gene

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Propylparaben case study - consumer exposure scenario

Report date 2025-09-03

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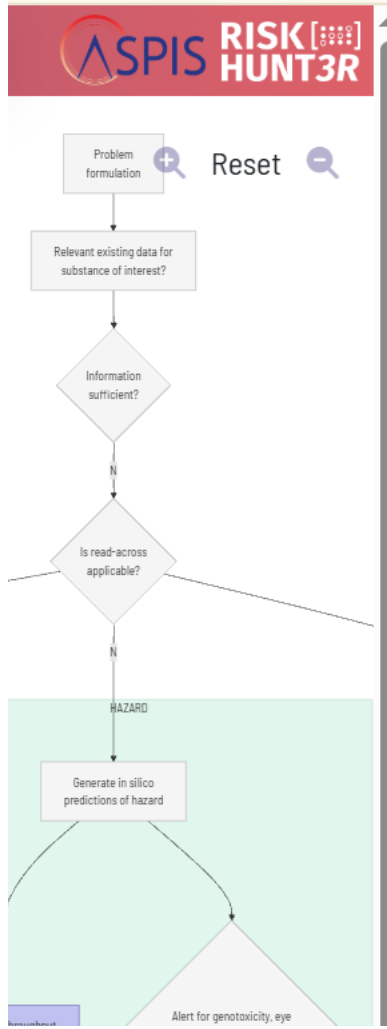

ASPA Handbook

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ASPA handbook

General | Hazard | ADME | Exposure | Integration

Problem formulation (BB001_01)

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)

Description | Proposed approach | Guidance | Connections

Enter a description of the problem formulation, which is 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

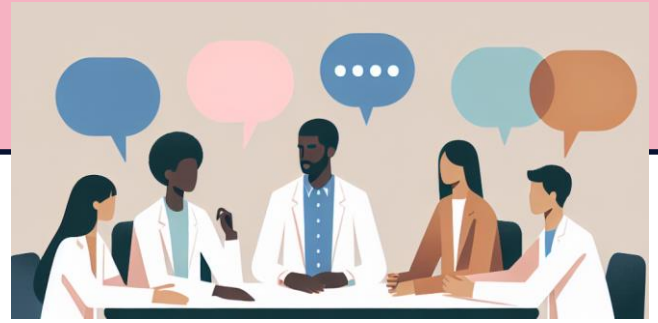
Provides decision criteria, which can be applied by the user (e.g. metabolite work flow)

What does it not do?

It is not a „one click NGRA prediction tool“

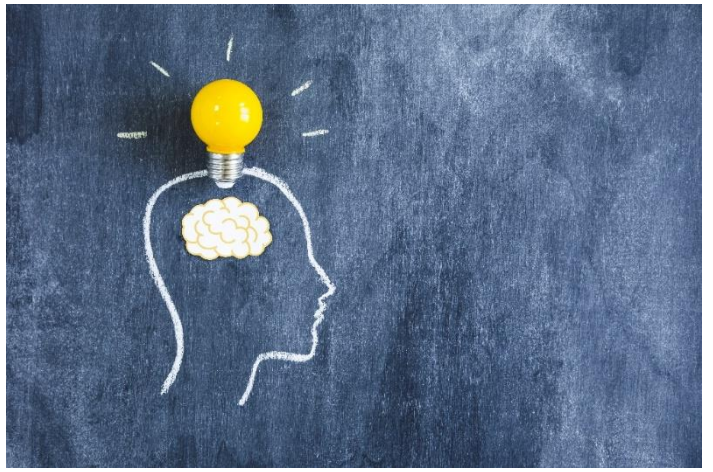
The user has to provide the data and assessments – thinking is ~~allowed~~ mandatory

The user has to take decisions



NGRA: Need to demonstrate applicability

- 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

Case studies: Example

- **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 (PPARα)
  - Well-known mode of action (MOA) for liver tumor formation in rodents

PPARα activation → increase in enzyme activity → increase in cell proliferation → increase in hepatocellular tumors

Problem formulation

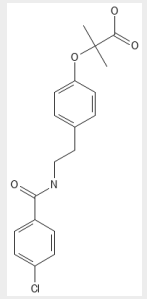
Key question:

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

Assumption is that existing data are insufficient or not available

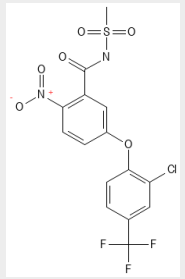
Carcinogenic substances

PPARα activation



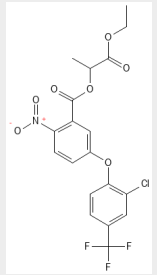
Bezafibrate

Liver; rats/mice



Fomesafen

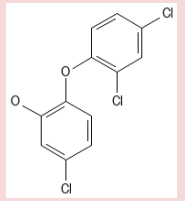
Liver; mice



Lactofen

Liver & stomach; mice

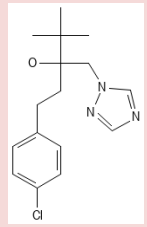
PPARα + CAR activation



Triclosan

Liver tumor; mice

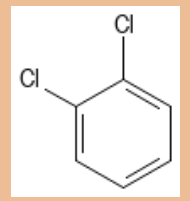
PPARα activation + other activities



Tebuconazole

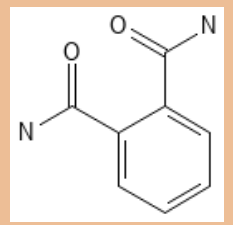
Liver tumor; mice

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

In silico & *in vitro* NAMs

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

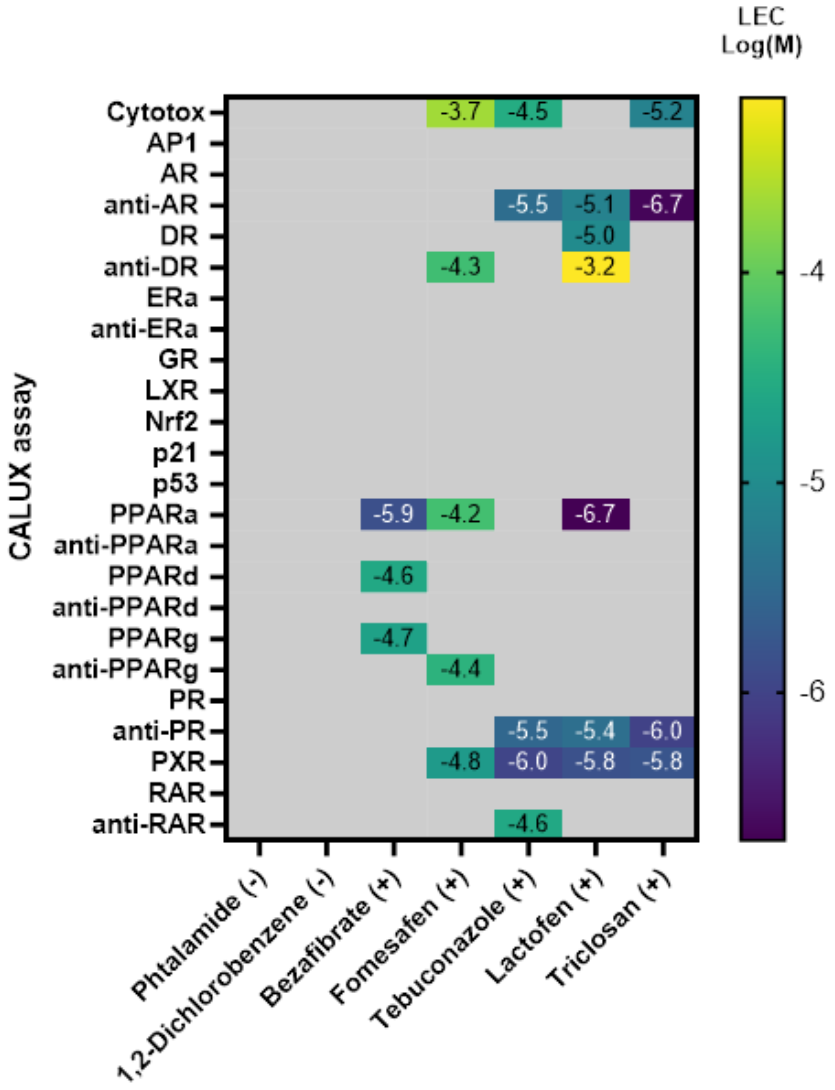
Toxicokinetics

- ADMET predictor
- PBK modelling

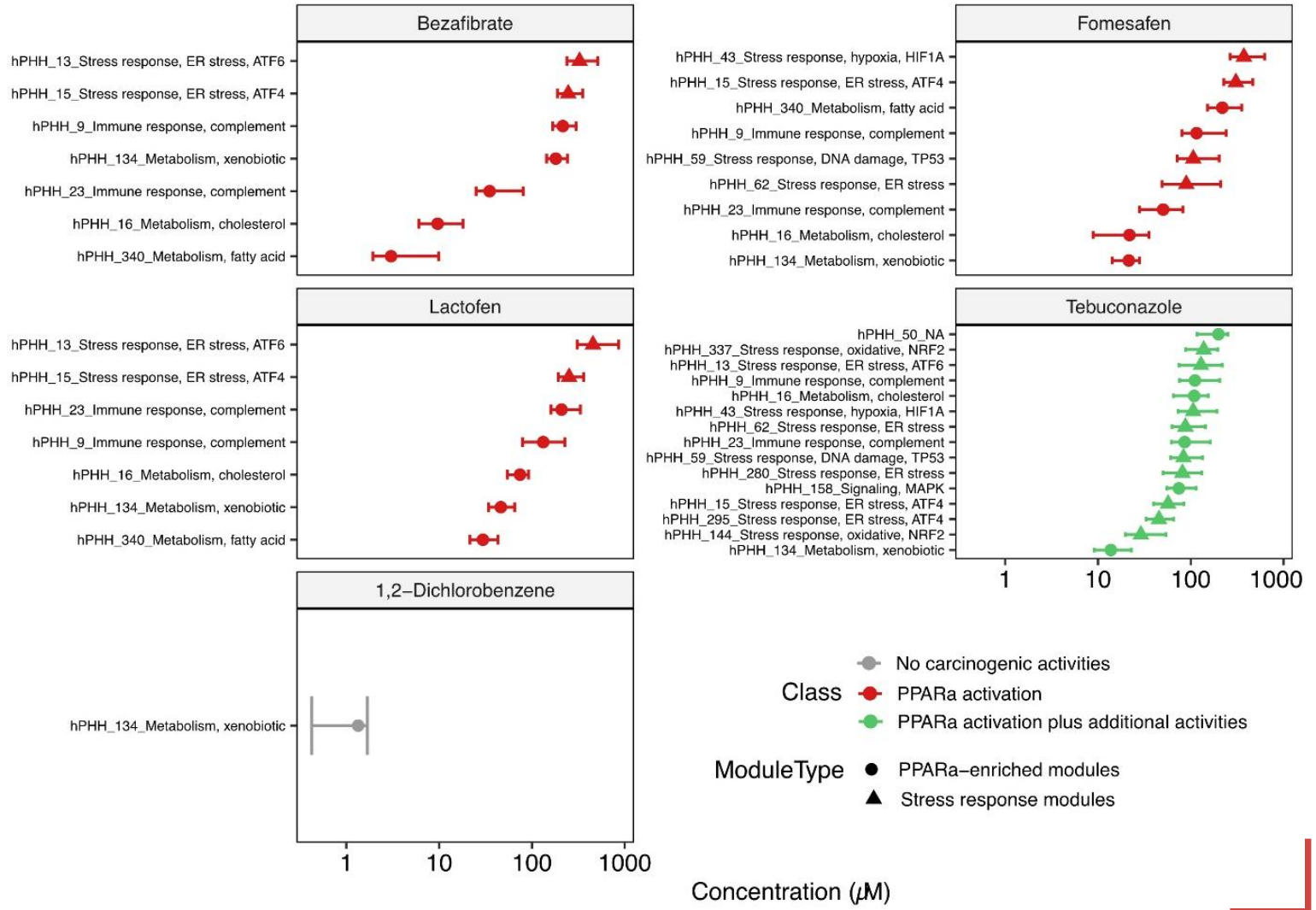
Should be considered as a case study for **tier 1** in ASPA

NAM Results: examples

CALUX



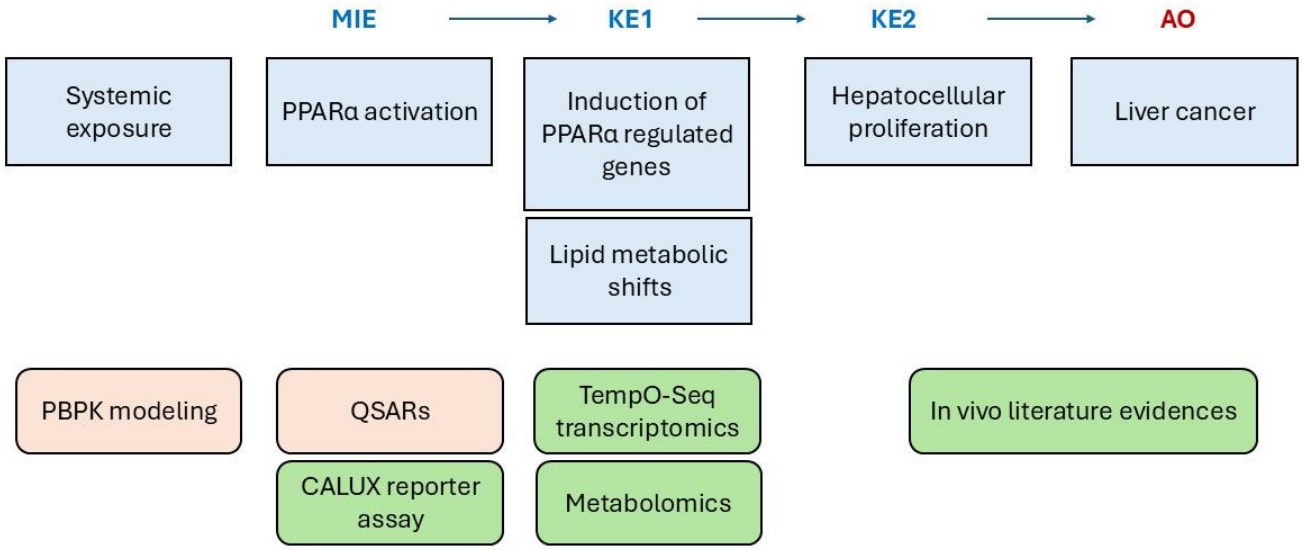
Transcriptomics in PHH



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



Case studies from RISK-HUNT*3R*

- 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

Towards the implementation of NGRA

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

Together, we'll make NGRA happen!


THANK
YOU