

Sustainability of ASPA beyond the project

As RISK-HUNT3R enters its final phase, we look back at what the project has delivered and how its results can continue to support the field beyond the project lifetime. A key outcome is ASPA, the interactive NGRA workflow that provides modules for toxicokinetic modelling, hazard identification, hazard characterization, exposure assessment and risk evaluation. Modules can be tailored to specific problem formulations, ensuring flexibility across the various regulatory contexts as well as safe-by-design use. Consistency, transparency, traceability and robustness are all key elements of ASPA to ensure that the same data always lead to reproducible conclusions.

ASPA was designed to be more than a project-specific deliverable. Its interactive structure helps both data producers and regulators navigate the risk assessment process, supporting transparent decision-making in various regulatory needs ([Leist et al. 2025](#); [Luijten et al. 2026](#)).

This is particularly relevant in the current European context, where the transition away from animal testing is becoming increasingly concrete and the need for reliable non-animal approaches continues to grow. The European Commission's roadmap towards phasing out animal testing for chemical safety assessments further underlines the relevance of RISK-HUNT3R, highlighting how the formulation of NGRA frameworks such as ASPA is "*an indispensable prerequisite*" for further work on the roadmap. It also reflects key project concepts, including mechanistic evidence and the integration of non-animal data, and helps clarify how future legislative and guidance developments could support the transition towards animal-free safety assessment.



From case studies to regulatory dialogue

Over the course of the project, RISK-HUNT3R developed a series of case studies to test and refine ASPA in practical settings. These case studies cover different regulatory scenarios and explore how new approach methodologies (NAMs) can be applied in practice, how evidence can be structured, how uncertainty can be addressed and how conclusions can be made more transparent and scientifically robust.

RISK-HUNT3R selected several case studies that have been submitted to the OECD under the Integrated Approaches to Testing and Assessment (IATA) case study framework. The first cycle of submissions was well received and generated positive feedback, confirming the value of structured workflows such as ASPA when applied to real case studies, in different regulatory-relevant contexts.

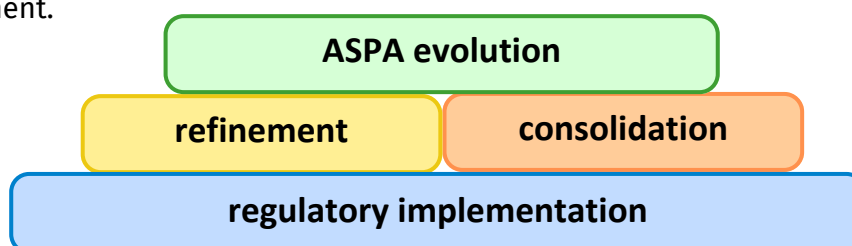
The first cycle of OECD-submitted RISK-HUNT3R case studies was introduced in Newsletter Issue #8, which provides an overview of the earlier case studies and their role in testing ASPA across regulatory contexts. Scan the QR code to read it.

In this issue, we present the second cycle of OECD-submitted case studies, with two new examples of ASPA application in practice.



Ensuring ASPA's sustainability requires continued maintenance and updates to the workflow, the assembly of increasingly sophisticated and challenging case studies, and keeping the approach aligned with ongoing NGRA and OECD developments.

The achievements of RISK-HUNT3R reflect the work of a broad and highly collaborative consortium. The project has relied on the contribution of many partners and disciplines. This shared effort provides a strong basis for continued progress towards next-generation chemical safety assessment.



"With all the different connections across the different modules, the ASPA workflow is a lot like a bowl of spaghetti. Lots of different strands, feedback loops, etc. ASPA-assist allows you to look at it as a much clearer, straightforward pathway – so you only see the piece of spaghetti you need to."

Nicholas Ball, Dow Chemical, member of the RISK-HUNT3R SRAB



Stakeholder Symposium: Algorithmically-structured NGRA. Helsinki, June 17-18, 2025

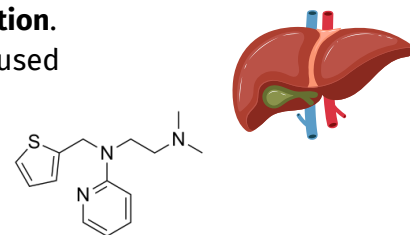
New case studies in 2026: ASPA for carcinogenicity assessment

1. Case study on *in vitro* transcriptomics for a mechanism-based carcinogenicity assessment

This case study explored how human-relevant, non-animal methods can help assess whether a substance may contribute to cancer development. Long-term rodent studies are time-consuming, resource-intensive and do not always provide clear insight into the biological mechanisms that are most relevant for humans.

As a proof of concept for a new approach, the case study focused on one specific pathway linked to liver cancer: **CYP2E1 activation leading to liver tumour formation.**

Methapyrilene, a substance known to cause liver tumours in rats, was used as the reference compound. The main question was whether *in vitro* transcriptomics could provide useful mechanistic information for carcinogenicity assessment.

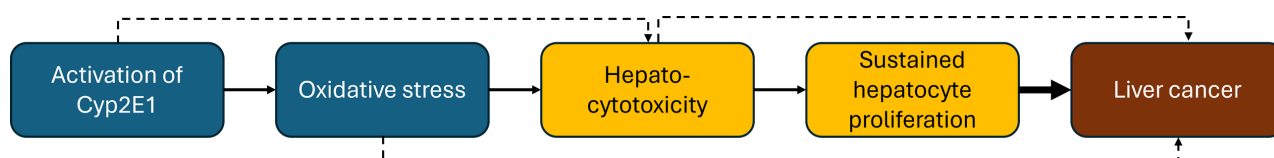


Following the ASPA workflow, the assessment was structured around the mechanistic question, linking the transcriptomics data with kinetic modelling and other relevant evidence.

To investigate this, researchers compared data from human liver microtissues exposed repeatedly to methapyrilene with existing data from short-term rat liver studies. Kinetic modelling was used to make sure that the exposure levels in the *in vitro* and *in vivo* studies were comparable.

The results showed a strong agreement between the human *in vitro* data and the animal data. Key biological responses linked to the selected pathway, including oxidative stress, liver toxicity, and changes related to cell proliferation, increased over time. This pattern is consistent with the build-up of hazardous effects after repeated exposure.

Overall, the case study shows that repeatedly exposed human liver microtissues, combined with transcriptomics and kinetic modelling, can provide valuable mechanistic evidence for carcinogenicity assessment. It represents an important step towards more human-relevant approaches and supports the broader move to reduce reliance on animal testing in regulatory decision-making.



AOP #220: Activation of CYP2E1 leading to liver tumour formation. Solid lines represent direct key event relationships (KERs) and dashed lines represent indirect KERs. Blue boxes represent molecular and cellular key events, yellow boxes represent tissue-level key events, and red represents the adverse outcome. Modified from Veltman et al. (2025).

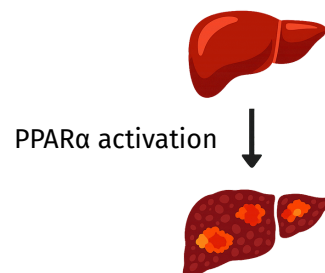
Elias Zgheib (Certara)



2. Case study on NAM-based approach for CLP-relevant assessment of carcinogenic potential

This case study examined how NAMs can help assess whether a substance may have carcinogenic potential in a way that is useful for classification under the CLP Regulation. Instead of relying only on traditional animal studies, the work explored whether non-animal data can provide early and meaningful information on the biological changes linked to cancer development.

The proof-of-concept case study focused on one well-described mechanism: liver tumour formation linked to activation of PPAR α , a receptor involved in the regulation of lipid metabolism and other liver processes. The aim was not to address all possible cancer mechanisms, but to test whether available *in silico* and *in vitro* methods could detect patterns of biological activity relevant to this specific pathway.



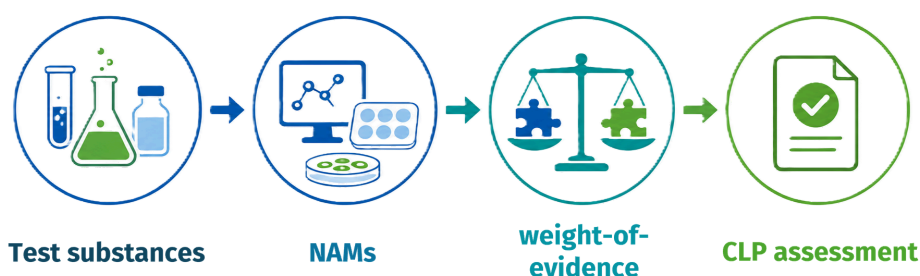
Following the ASPA workflow, the assessment was structured around the CLP-relevant question, bringing together information from different NAMs and evaluating it through a transparent weight-of-evidence approach.

Seven substances were included in the assessment: five substances known to cause liver tumours in rodents and two substances not considered carcinogenic.

The results showed that the substances associated with liver tumour formation produced biological response patterns that could be distinguished from those of the non-carcinogenic substances. This suggests that NAM-based data can help identify mechanistic signals relevant to carcinogenic potential, especially when interpreted together with supporting evidence, such as information from repeated-dose toxicity studies where available.

Overall, the case study shows how ASPA can support a more transparent and structured use of NAMs in carcinogenicity assessment. It also highlights the next challenge: expanding this type of approach to cover a broader range of mechanisms relevant to cancer development in humans.

Swapnil Chavan (RISE)



We encourage stakeholders to explore and test the ASPA workflow with new case studies to support broader uptake of NGRA approaches.

We offer training and access to the supporting software platform ASPA-assist.

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