THIRD YEAR ANNUAL REPORT FOR ASPIS

(REPORTING PERIOD: NOVEMBER 2023 TO NOVEMBER 2024)

TABLE OF CONTENTS

FIGURES & TABLES

EXECUTIVE SUMMARY

ASPIS is a confluence of three Horizon 2020-funded projects: *PrecisionTox*, *ONTOX* and *RISK-HUNT3R*. Its goal is to unite the three consortia, each with its own distinct yet mutual conceptual frameworks to integrate mechanisms of chemical toxicity with innovative methods to assess and regulate hazardous chemicals without traditional toxicity testing using animals. During its third year, ASPIS continued to collaborate, learn and advance regulatory science through its working groups (WG). The eight WGs and the ASPIS Academy are defined by their specialised areas of research and activities applied towards the development of new approach methodologies (NAMs). Aligned with the ASPIS Academy is the continued development of an ASPIS sustainability plan, as well as an evaluation of its impacts in risk and hazard assessment. The overall aim is to ensure that ASPIS investments in NAMs, produce lasting information resources for the regulatory community.

During its third year, ASPIS focused on the continued development of the ASPIS-initiated Safety Profiling Algorithm (ASPA), which encompasses the ASPIS next generation risk assessment workflow, and related concepts. The continued development of ASPA, as well as WG-specific projects focused on the study of chemicals leading to steatosis and developmental neurotoxicity. Additionally, a chemical group-focused study on the conazoles was initiated. The three groups of substances were chosen to exploit the diversity of ideas and approaches of all three projects, by sharing data produced from a shared set of study chemicals, which are also prioritised by our stakeholders.

Achievements of Year 3 include:

- An ASPIS consensus definition of the meaning of NAMs.
- An ASPA version 2.0: a framework for the integration of NAM data for the hazard and risk assessment of chemicals.
- Participation in 3R's-oriented discussions and events organized by the European Commission.
- Dissemination of results in several international and national conferences.
- Organization of a hackathon and other events involving the general public.
- Organization of several ASPIS Academy activities.
- Teaming up with other relevant groups and consortia to leverage impact.
- Development of an AI-driven platform serving as a co-pilot to predict chemical-induced toxicity.
- Publication of AI-optimized Adverse Outcome Pathway (AOP) networks for chemical-induced steatotic and cholestatic liver injury.
- Set-up of an AOP-anchored *in vitro* testing battery to predict chemical-induced cholestatic liver injury.
- The dissemination of ASPA to the general public through meeting presentations (platform, posters) and workshops.
- Continued development and release of knowledge resources including database infrastructure.
- Publication of an ASPIS NAM definition.
- Dissemination of WG products via meeting presentations, article publications, media release, webinars and educational events.

The outlook for 2025 includes the additional peer-reviewed publications from investigations of the WGs, a growing list of opportunities for its ESRs, implementation of its sustainability plans and a continuing focus on the co-development of ASPA and related concepts. Additionally, ASPIS will continue to strengthen current and develop new connections among ASPIS and other major initiatives focused on the implementation of the 3R's in hazard and risk assessment.

BACKGROUND

ASPIS is a collaboration of the Horizon 2020 funded projects *ONTOX*, *PrecisionTox* and *RISK-HUNT*3R. It represents Europe's €60M effort towards the sustainable, animal-free and reliable chemical risk assessment of tomorrow. ASPIS includes more than 70 institutions across 16 countries of the European Union plus the United Kingdom and the United States. The mission of ASPIS is **to establish a next-generation risk assessment framework based on new approach methodologies (NAMs), encompassing *in vivo* to *in silico* technologies**.

ASPIS maintains its commitment to improving the accuracy, speed and affordability of chemical safety testing without the use of laboratory animals. Building on advances within the three consortia and close interactions with its stakeholders, it provides NAMs to accelerate and improve chemical risk assessment. Through these activities, informed decisions that safeguard human and environmental health can be made to facilitate the development of safe and sustainable products. The goals of ASPIS are being met through international cooperation, methods development and guidance documents for chemical safety testing and risk assessment.

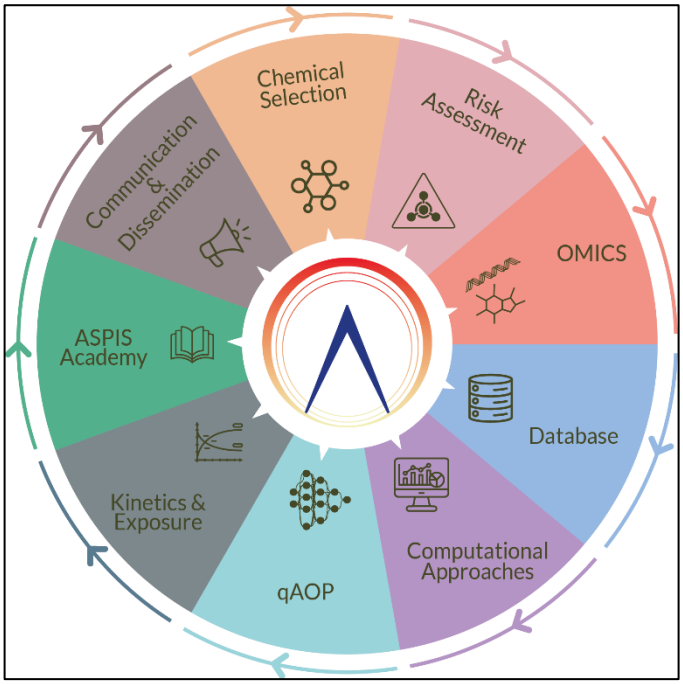

Figure 1. ASPIS Working Groups

ASPIS collaborations function through eight Working Groups (WGs); Chemical Selection, Risk Assessment, quantitative Adverse Outcome Pathways, Omics, Kinetics & Exposure, Computational Approaches, Database, and Communication & Dissemination: and the ASPIS Academy, which are composed of investigators from all three consortia that specialise in activities that are relevant to the cluster's mission (Figure 1).

The WGs are co-chaired and include members from each of the three projects, the ASPIS Academy and the Joint Research Centre (JRC). WGs have regularly scheduled monthly or bimestrial virtual meetings, as well as *ad hoc* meetings when needed. Meeting recordings and other ASPIS documentation (manuscripts, posters, PowerPoint presentations and meeting slide decks) are maintained on the ASPIS MS Teams/SharePoint. Additionally, there are dedicated Slack, GitHub, LinkedIn and several web sites.

The ASPIS coordination team is composed of the three ASPIS projects' coordinators: Bob van de Water, Leiden University, RISK-HUNT*3R*; John Colbourne, University of Birmingham, PrecisionTox, and Mathieu Vinken, Vrije Universiteit Brussel, ONTOX; the three ASPIS project managers (Martijn J. Moné – RISK-HUNT3R, Agata Ormanin-Lewandowska – PrecisionTox, Julia D. Zajac – ONTOX) and the Working Group Coordinator – Jonathan Freedman (PrecisionTox).

The ASPIS project managers are crucial for ensuring smooth coordination and effective communication across the consortia. They assist the coordinators in efficiently achieving the ASPIS goals and their responsibilities include organizing coordinators' meetings, compiling meeting reports and facilitating cross-communication between the three ASPIS projects, coordination team and the working group coordinator (WGC). Additionally, they are instrumental in organizing the ASPIS Open Symposia.

The ASPIS Working Group Coordinator (WGC continues to play a critical role in the development and reporting on ASPIS inter-working group projects, as well as a link among the WGs, ASPIS leadership

and Scientific/Regulatory Advisory Boards. Furthermore, the WGC communicates WG activities to the international community through their attendance and presentations at international meetings, EU workshops and publications.

ASPIS-WIDE ACTIVITIES

Successes and Opportunities

ASPIS launched in July 2021 under the leadership of Bob van de Water, Leiden University, RISK-HUNT*3R* coordinator. The ASPIS leadership role was then inherited by John Colbourne, University of Birmingham, PrecisionTox coordinator. At this year's ASPIS Open Symposium, leadership was transferred to Mathieu Vinken, Vrije Universiteit Brussel-Belgium, ONTOX coordinator.

During the first year of ASPIS, formal organisational and WG structures were established. Its mission and goals were developed and areas for cross-consortium collaboration were identified. In its second and third years, ASPIS built on this foundation with each WG pursuing its individual activities, as well as several ASPIS-wide projects. The final years of ASPIS will focus on communicating and implementing discoveries made by the cluster. Additionally, cluster members will discuss and implement approaches to increase ASPIS impact and sustainability.

Organisational meetings continue to be held on a regular basis including:

- Coordinators meeting (monthly) is attended by the ONTOX, PrecisionTox and RISK-HUNT*3R* coordinators, project managers and the WGC. Additionally, representatives from the European Commission (EC) are invited to attend.
- WG chairs meeting (monthly) includes consortia coordinators, project managers, WGC, chairs of each WG and the ASPIS Academy Core team.
- Stakeholder and Regulatory Advisory Board (SRAB meeting (quarterly) is attended by the five members of the SRAB, project coordinators, project managers and the WGC.
- *Ad hoc* meetings are held at various times throughout the year. During this reporting period ASPIS coordinators, project managers and the WGC met with members of PARC, EPAA, EFSA, VICT3R and the US FDA, EPA and NIEHS.

ASPIS continued to expand its efforts in the steatosis case study. Each WG has been actively engaged in this case study. Several of the WG are preparing or have already submitted manuscripts based on their work as part of the case study. Details of each WGs contribution to the steatosis case study can be found below.

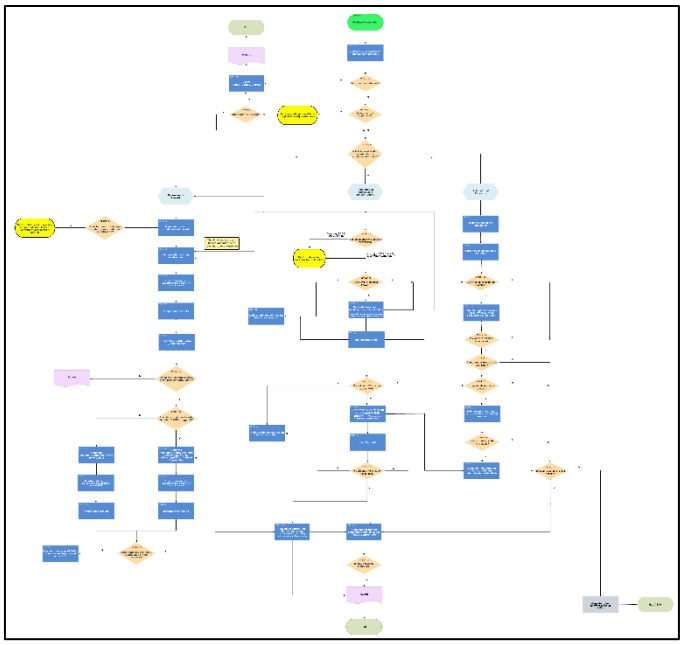

Figure 2. ASPA Workflow Version 2.0

The second ASPIS-wide project is the continued development of the ASPIS Next Generation Risk Assessment (NGRA) framework. This framework, called the ASPIS Safety Profiling Algorithm (ASPA), is a tiered approach to identify tools and methods, when to obtain and evaluate data and how to put data into a context of a hazard or risk assessment scenario. ASPA defines a decision logic with multiple entry and exit points, activating or deactivating specific modules and prioritising and filtering of information (Figure 2). The steatosis case study served as the basis for the early development of ASPA. A smaller case study was initiated focused on a collection of developmentally toxic conazoles. A list of conazoles, metabolites and control compounds and their cognate physical-chemical and toxicological data has been

collected by the CSWG. This information has been distributed and is being used in the continued development of ASPA.

ASPA has extensively been presented and discussed at several international meetings as stand-alone platform presentations and posters, as well as portions of broader ASPIS presentations. It was the centrepiece of the public section of the 4th Annual ASPIS Open Symposium (Copenhagen, Denmark September 2024). Additionally, ASPA has been presented at several international meetings and workshops, including the EC workshop on the roadmap on phasing out animal testing for chemical safety assessment in Brussel, Belgium in November 2024.

The ASPA framework will become publicly available before the end of ASPIS; meanwhile intermediate versions will be communicated with all critical stakeholders. A dashboard (i.e. electronic) version to be accessible via a web page to facilitate application is being developed and currently applied. It will provide a formal NAM-based process for the safety assessment of chronic adverse health effects associated with chemical exposure. An ASPIS workshop dedicated to the implementation of ASPA will be held at the German Federal Institute for Risk Assessment (BfR) in May 2025.

ASPIS-wide Challenges

The critical challenge remained the lack of dedicated funds for ASPIS activities. All ASPIS activities fall within the goals and deliverables for each project. The lack of independent funds limits the range of ASPIS activities, especially those of the ASPIS Academy. All ASPIS activities are being performed by its members, in addition to their consortium-specific duties (e.g., meetings, logistic and work packages activities), on a voluntary basis.

The second challenge faced by ASPIS and its three consortia is sustainability. A major concern is the loss of accumulated knowledge, data, SOPs, etc. when cluster funding ends. This concern is shared by members of our advisory board and stakeholders. The topic of sustainability will be one of the main foci for this coming year.

An even more prominent discussion on the application of NAMs and NGRA has appeared in the toxicological science and regulatory/policy communities. However, NAMs and NGRA are not universally accepted in risk assessment and regulatory communities and their adaption is slow in coming. NAMs and NGRA are components of dynamic fields with constantly changing needs and goals from stakeholders and the regulatory community. Last year we noted that a single position regarding the definition of NAMs was not yet established by ASPIS. To address this issue, a small work group prepared a draft document and presented it as a poster at the 3rd and 4th Annual ASPIS Open Symposium for external input. Based on this input, a manuscript was and now in press in a special issue of ***Environmental Toxicology & Chemistry*** entitled: ***New Approach Methods (NAMs) in Ecotoxicology*****.**

Aims for 2025

ASPIS will continue and expand its interaction with other EU, UK and US projects focused on implementing the 3R’s in hazard and risk assessment. It will continue to expand its international presence and champion the acceptance of NAMs by presenting at local, national and international meetings. It will also participate workshops focused on NGRA and NAMs, organising workshops, teaching and meeting sessions. Additional activities are presented below by the Communication & Dissemination WG.

ASPIS will continue to prepare and submit ASPIS-wide publications. Several WGs are currently preparing manuscripts that will include members from the three consortia and multiple WGs. These manuscripts will be submitted for publication during the coming year. Additionally, several consortium-specific publications are being prepared. These publications, however, will include input from members from various ASPIS WGs. In addition to publications, ASPIS will utilise available opportunities to

present its work at workshops, symposia and regional, national and international meetings and submit meeting session proposals.

At the 4th Annual ASPIS Open Symposium, the WGs chairs and leadership held discussions on impact and sustainability. Additionally, necessity of the WGs, as well as their roles in addressing these topics was discussed. At the conclusion of the meeting, the following recommendations were made:

- Form three task forces, each focused on a specific topic
    - ***ASPA***
    - ***Sustainability***
    - ***Impact***, as defined in the original H2020 call:
        - Scientifically sound, practicably implementable non-animal solutions readily deployable to aid in meaningful safety assessment of chemicals.
        - Recognition from regulatory bodies and their engagement to translate results, methods and solutions into safety assessment practice.
        - Uptake and commercial exploitation of the developed safety assessment approaches, products and services.
        - Contribution to the 3Rs principles ('Replacement', 'Reduction', 'Refinement'), with a particular emphasis on the 'Replacement' opportunities.
- Each WG will have the option to remain, participate in the task forces or end its activities. The status of each WG is presented below.

Dissemination Activities

- Oral Presentations; *Good practices and resources to improve the utility of research data in regulatory assessments*, European Commission's Joint Research Centre, online webinar.
- Exhibitor's booth; 63rd Annual Meeting and ToxExpo of the Society of Toxicology, Salt Lake City, Utah, USA.
- Oral and Poster Presentations; 58th Congress of the European Societies of Toxicology, Copenhagen, Denmark.
- Oral Presentation; 2nd Conference on the Commission Roadmap towards Phasing Out Animal Testing for Chemical Safety Assessments, Brussels, Belgium.
- Exhibitor's booth; 58th Congress of the European Societies of Toxicology, Copenhagen, Denmark.
- Published; ASPIS NAMs definition paper entitled, *ASPIS Definition of New Approach Methodologies* by John Colbourne, Sylvia Escher, Robert Lee, Mathieu Vinken, Bob van de Water and Jonathan Freedman.
- Contributed sections; 2023 *Non-Animal Methods in Science and Regulation*; EURL ECVAM status report.
- ASPIS contributed/joined PARCopedia.
- Additional dissemination activities are presented by each WG below.

INDIVIDUAL WORKING GROUP ACTIVITIES

ASPIS Academy (AA)

Co-chairs: Eliska Kuchovska (ONTOX), Luiz Ladeira (ONTOX), Barira Islam (RISK-HUNT*3R*), Kirsten Veltman (RISK-HUNT*3R*), Ruben Martinez (PrecisionTox), Gaelle Hayot (PrecisionTox), Shaleen Glasgow (PrecisionTox), Julen Sanz Serrano (ONTOX), Marie Corradi (ONTOX), Peter Pobis (ONTOX), Rita Ortega Vallbona (ONTOX), A. Melina Steinbach (RISK-HUNT*3R*), Eike Collen (RISK-HUNT*3R*) and Hiba Khalidi (RISK-HUNT*3R*).

Background

The ASPIS Academy (AA) is a network for early-stage researchers (ESRs) focused on the development and use of NAMs for chemical risk assessment. The AA was established in the second half of 2023 with the goal of promoting the careers of ESRs by providing specialized training and equal opportunities for its members and creating a platform devoted to the voices and aspirations of a new generation of young scientists who will become experts and future leaders of NAMs and their applications. The AA is led by the AA core group composed of ESRs representing the three projects and advised by AA Board composed of the three ASPIS project managers (Agata Ormanin-Lewandowska - PrecisionTox, Julia D. Zajac - ONTOX and Martijn J. Moné - RISK-HUNT3R) and AA advisors (François Busquet, Helena Kandarova, Silvia Tangianu).

Successes and Opportunities

The year 2024 has been very successful for the AA. Thanks to the numerous activities organized for ~ 130 ASPIS ESRs. Additionally, the AA has opened some of its programs (especially the online trainings) to external ESRs. They were invited to join the *AA LinkedIn group*, where they are informed about AA activities. The AA organized two career development sessions in 2024, inviting eight guests with different career paths to give insights into their roles. The first session involved guests representing academia, industry, intergovernmental organization and an NGO, while the *second session* featured a forensic toxicologist, senior publisher, consultant and senior project manager. Three additional online training sessions were organized focusing on the topics: *Connecting with Your Audience*, *Finding European Research Funding and Writing a Competitive Research Proposal* and *The Importance of Science for Policy and Science Communication for 21st Century Toxicology*. The AA also organized one in-person training at the ASPIS OS in Copenhagen featuring three speakers discussing *How to Transform Your Research into a Spin-Off Business* (Figure 3).

Figure 3. In-person training "How to transform your research into a spin-off business" at the ASPIS Open Symposium in Copenhagen in September 2024.

The ASPIS OS in Copenhagen allowed for more in-person activities including an AA poster session, presentation and networking activities including a sightseeing walk through Copenhagen and a pub quiz (Figure 4). Some of the trainings organized by the AA have been recorded and can be found on the *ASPIS YouTube channel.* The highlight of the year was a *scientific session organized by AA at the ESTIV congress* in Prague (Figure 5). The session featured one presentation introducing the AA and three scientific presentations of selected ASPIS ESRs, one from each consortium.

Figure 4. Networking evening of the ASPIS ESRs in Copenhagen

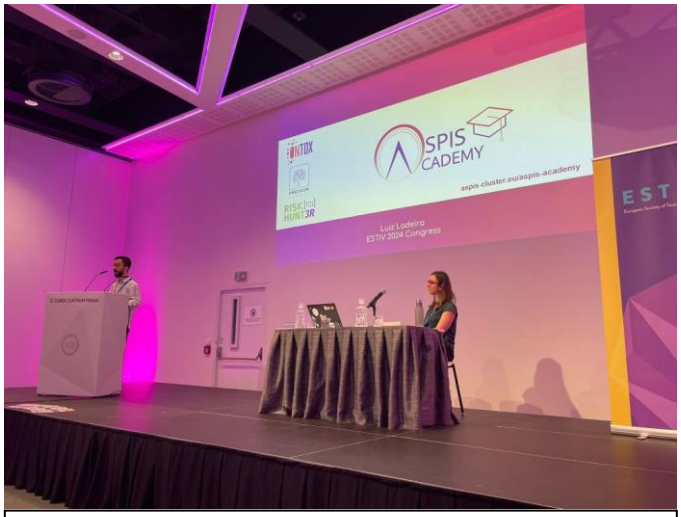

Figure 5. Scientific session organized by the ASPIS Academy at the ESTIV Congress in Prague in June 2024.

ESRs have been trained in conducting interviews of which *four were already published on the AA webpage*. The AA webpage has been extensively updated and expanded to include subsections about the *AA core group members*, *AA news* and a *subsection introducing the AA* featuring an introductory video. The AA has been working on its dissemination activities using various tools such as *videos*, posters at scientific conferences. The AA is also active at ASPIS and Altertox booths at conferences offering materials such as AA flyers, business cards and stickers. The AA has successfully carried out the first round of its mentoring program featuring ten mentorship pairs with the second round of applications planned for 2025. ASPIS ESRs joined the mirroring program bringing ESRs to ASPIS WGs to learn the necessary skills and obtain scientific and collaboration opportunities. The AA actively engaged in external collaboration opportunities by contacting and organizing joint activities with the *PARC Junior Community, young TPI, SETAC Student Advisory Council, ESTIV Early Career Network* and *EUROTOX Early Career Forum*.

Shortcomings and Uncertainties

AA activities are dependent on budget availability. Thus, the scope and number of activities rely on potential financial support from sponsors. Therefore, the Twinning program of the AA, which aims to facilitate short internships of ASPIS ESRs in ASPIS partner laboratories and companies, has seen only one ESR participating in this program.

Aims for 2025

Numerous activities are being prepared for 2025. First, the AA summer school is planned to take place in Valencia in April as a satellite event of the ONTOX annual meeting. Poster sessions, in-person training and networking activities are also envisaged for the ASPIS OS in Athens in September.

The AA submitted two session proposals for conferences: 13th World Congress on Alternatives and Animal Use in the Life Sciences and SETAC Europe. Additional AA sessions might take place in 2025 following the successful example of the ESTIV session in 2024. The AA plans to continue with its career development activities in the training, mentoring and twinning programs. Additionally, four interviews with AA speakers are now being conducted and will be published in 2025. The AA core group is preparing a scientific publication about AA; its creation, goals and legacy with an expected publication date in the first half of 2025. The year 2025 will especially mark AA's focus on its sustainability and network legacy. Finally, in 2024, most of the original core group members left AA and new ESRs from the three projects joined the efforts (both original and new members are listed as co-chairs above). Their new fresh ideas might give rise to new additional AA activities.

Impact

The AA is contributing to the development of non-animal safety assessment approaches, aligning with the 3Rs principles, with a strong focus on 'Replacement'. The AA training programs help ESRs to develop and use innovative methods to replace animal-based approaches in safety assessments. AA also helps ERSs develop critical skills to align their work with regulatory demands, thereby encouraging the adoption of these approaches by industry and regulatory bodies. Finally, the AA focused efforts on sharing outcomes of ASPIS ESRs via different communication and dissemination tools and conference participation are designed to encourage the widespread adoption of developed methods, further amplifying their impact and commercial viability. Through these measures, the Academy's activities align with the goal of implementing sustainable and innovative safety assessment approaches.

Status

The AA will continue its activities.

Chemical Selection Working Group (CSWG)

Co-chairs: Sylvia Escher (RISK-HUNT*3R*), Jonathan Freedman (PrecisionTox) and Mathieu Vinken (ONTOX)

Background

The goal of the Chemical Selection Working Group (CSWG) is to coordinate activities associated with chemical selection and cognate data among the ASPIS partners. Members of this WG are responsible for collecting and distributing information on chemicals being used among the three consortia. Through its coordinated effort, the CSWG minimises duplication of efforts and identifies inter-consortia activities. The CSWG is involved in the development of ASPIS-wide case studies and assisting other WGs by providing physicochemical and toxicological information on individual or groups of chemicals. Additionally, the CSWG serves as a link to other 3R's and toxicological projects in the US, UK and EU to identify inter-project activities.

Successes and Opportunities

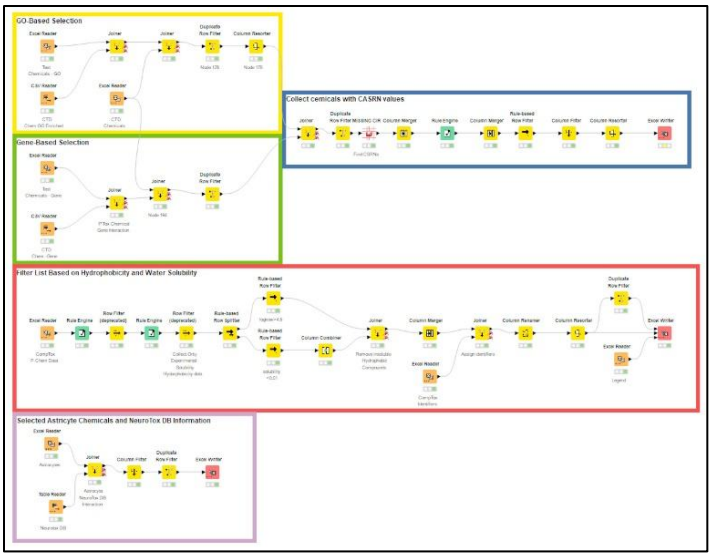

Figure 6. Requested KNIME Workflows to identify Astrocyte-specific toxicants

Most of the chemical selection and testing among the three consortia have been completed or are well underway. For these reasons, the activities of the CSWG during this reporting period have been reduced to data collected and distribution. Specific request for chemical data including physicochemical properties, toxicological information, vendors/sources and selection protocols have been provided. This information has been provided to all WGs and is to be included in the ASPIS database. The CSWG is supporting the development of the ASPA by providing physicochemical, toxicological and exposure information to the Risk Assessment WG. To expedite data mining for specific requests the CSWG created several KNIME workflows to collect information from public and private databases (Figure 6).

Shortcomings and Uncertainties

Since all ASPIS chemicals have been selected, the remaining activities of the CSWG have been reduced to a level that can be accomplished by a few persons. For this reason, the CSWG will be integrated into the DbWG. Future requests for chemical information will be addressed by the ASPIS Coordinator.

Aims for 2025

The CSWG will continue to work with the JRC on the creation of an ASPIS chemical library. These chemicals will be made public and provided to interested investigators with the goal of expanding toxicological information on the ASPIS compounds. Based on a PrecisionTox manuscript on chemical selection, due for submission in January 2025, the ASPIS CSWG will begin preparing a manuscript on protocols used by consortia members for their chemical selection.

The CSWG will continue to help building the ASPIS database with the DbWG. It will also support the Risk Assessment WG in the development of ASPA. Additionally, it will assist other WGs regarding issues associated with chemical selection and characterization. Finally, several groups concerned with the 3R's have approached ASPIS for chemical information including PARC, the JRC and the 3Rs Collaborative.

Dissemination Activities

- Posters at the 4th ASPIS Open Symposium Copenhagen, Denmark.
- Provided ASPIS chemical lists to interested parties in the EU and US.
- The majority of the CSWG Dissemination Activities are incorporated into the activities of other WGs.

Impact

The CSWG has coordinated the methods employed by the three consortia for chemical selection and supplied chemical information to a searchable database. The methods and database could become resources to aid in chemical safety assessment.

Status

The CSWG is being incorporated into the DbWG.

Communication and Dissemination Working Group (C&DWG)

Co-chairs: Helena Kandarova (ONTOX), Francois Busquet (PrecisionTox) and SilviaTangianu (RISK-HUNT*3R*)

Background

The Communication and Dissemination Working Group (C&DWG) aims to harmonise dissemination activities and maximise the impact of ASPIS. Communication and dissemination activities are coordinated by the three consortia with the shared mission to inform unbiasedly on how NAMs-based strategies can rapidly accelerate and improve chemical risk assessment without the use of animals.

Communication teams of the three consortia work together to synergise dissemination efforts and positively affect ASPIS visibility and the outreach of its key messages. In particular, the goal of the C&DWG is to build, beyond projects' specificities, a single ASPIS to have a stronger voice to vehicle its outcome to regulatory stakeholders, policy makers, non-governmental organisations and the lay public.

ASPIS speaks jointly online via a coherent social media presence to facilitate message amplification. The communication teams of each consortium echo news and outcomes of each other and disseminate those via their channels. ASPIS also jointly communicates via official press declarations and policy briefs, as support statements of policy legislative initiatives or involvement in public consultations. Common publications, op-eds and press coverage in European journals are also used to inform stakeholders about ASPIS.

The WG organizes also face-to-face activities, such as participation in joint conference sessions at international conferences (e.g., SOT annual conferences, EUROTOX) and the organisation of the ASPIS annual open symposium. The symposium series involves consortia members, stakeholders and the ASPIS SRAB to discuss crucial achievements and challenges in the implementation of NAMs into chemical risk assessment in Europe and beyond. This allows ASPIS to support EU Green Deal objectives, such as a toxic-free environment.

Successes and Opportunities

Building on the achievements of previous years, the C&DWG expanded its activities in 2024:

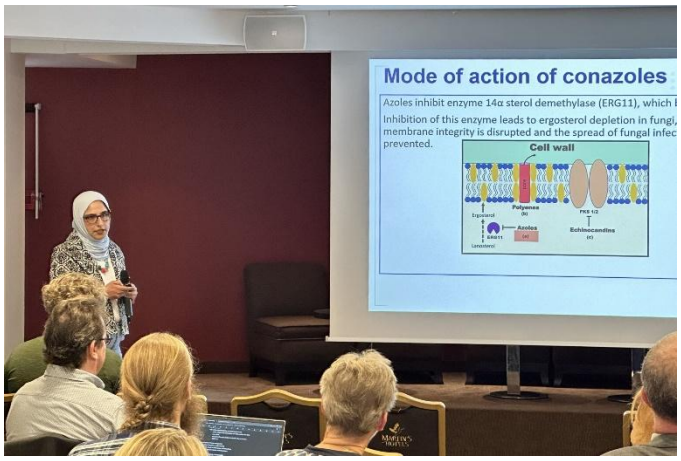

Figure 7. Barira Islam (RISK-HUNT*3R*) presenting at the 2nd European Commission workshop on the Roadmap Towards Phasing Out Animal Testing in Chemical Safety Assessments.

- **Stakeholder Engagement and Outreach:**
  - Actively reached out to stakeholders and regulators by organising scientific exchanges at the 2024 SOT ToxExpo (March 10–14, Salt Lake City, Utah, USA).
  - Participated in the EPAA Designathon co-hosted with JRC (20-22 March, Ispra, Italy).
  - Presented the cluster's work at the 3Rs working party of the European Medicines Agency (Online, 20 March 2024).
  - Attended the Helsinki Chemical Forum with a poster (5-6 September, Helsinki, Finland)
  - Presented ASPIS at a satellite event of the 2nd Workshop of the EC roadmap to phase out animal testing (October 25, Brussels, Belgium) (Figure 7) and the EPAA NAM user forum at ECHA in Helsinki (30-31 October 2024, Helsinki, Finland).
- **Communication and Dissemination Materials:**
  - Produced video interviews of ASPIS WP leaders at the SOT (10-14 March, Salt Lake City, Utah, USA) and the ASPIS OS Symposium in Copenhagen (11-12 September, Copenhagen, Denmark),
  - Reorganized and updated the ASPIS website to improve accessibility and dissemination.
- **Support for ASPIS Academy:**
  - Continuously supported ASPIS Academy activities, including mentoring, mobility facilitation and event organization.
  - Negotiated, organised and supported conference sessions, such as the ASPIS Academy Inaugural Meeting at ESTIV (June 3-6, Prague, Czech Republic), poster sessions at ASPIS OS (11-12 September, Copenhagen, Denmark).
- **Major Scientific Events:**
  - ASPIS was represented at key conferences such as US SOT (10-14 March, Salt Lake City, Utah, USA), ESTIV (June 3-6, Prague, Czech Republic) and EUROTOX (8-11,

September, Copenhagen, Denmark). These events highlighted ASPIS contributions to advancing NAMs.
    - Organized poster sessions and workshops, including science communication, grant writing and career development.
- **Broader Collaborations:**
    - Initiated collaboration with ESR communities, such as PARC Junior Community, SETAC SAC and Young TPI, to expand the impact of ASPIS initiatives.
- **Presence in the major meetings with the ASPIS booth**
    - ASPIS exhibitor booth at 63rd Annual Meeting and ToxExpo of the Society of Toxicology (Salt Lake City, Utah).

Aims for 2025

In 2025, ASPIS aims to maintain a strong presence at multiple scientific events, including the SOT, EUSAAT, SETAC and EUROTOX annual meetings. Activities will include scientific presentations, poster sessions and exhibitor booths. The C&DWG will coordinate the participation of cluster partners, disseminate scientific contributions across diverse programs and strengthen collaborations with EU institutions. Additionally, the C&DWG will support the organisation of the next ASPIS Open Symposium, scheduled to take place in Athens, Greece, immediately following the 59th Annual EUROTOX meeting. The C&DWG will also continue to support training events for the ASPIS Academy, including the Summer School planned during the ONTOX annual meeting in Valencia, Spain, in April 2025. Discussions are ongoing about organising events in collaboration with external initiatives including VICT3R, PARC, VHP4Safety and Young TPI.

Dissemination Activities

- ASPIS speakers at the EC 'Next Gen Chemical Safety Assessment' conference, Brussels, Belgium; March 4-6, 2025,
- ASPIS booth at the 64th Annual Meeting and ToxExpo of the Society of Toxicology, Orlando, Florida, USA; 16-20 March 2025,
- ASPIS Academy summer school, Valencia, Spain; 25 April 2025,
- ASPIS booth at the MPS Summit 2025, Brussels, Belgium; June 9-13, 2025,
- ASPIS speakers and booth, 13th World Congress on Alternatives and Animal Use in the Life Sciences, Rio de Janeiro, Brazil; 31 August - 4th September 2025
- ASPIS speakers and booth at 59th Congress of the European Societies of Toxicology, Athens, Greece; 14-17 September 2025,
- Fourth ASPIS Open Symposium, Athens, Greece; 17-18 September 2025.

Impact

The C&DWG has played a pivotal role in enhancing the visibility, outreach and impact of ASPIS. Through strategic communication efforts and harmonized dissemination activities across the three consortia, the C&DWG has established a strong presence in the scientific, regulatory, and public domains. The C&DWG proactively engaged with regulators, policymakers, and other stakeholders to advocate for the adoption of New Approach Methodologies (NAMs) in chemical risk assessment, presented ASPIS work at high-profile events, including the Society of Toxicology (SOT) Annual Meeting on 10–14 March 2024, in Salt Lake City, Utah, EUROTOX Congress on 8-11 September 2024 in Copenhagen, Denmark, and the European Commission's workshops on phasing out animal testing, supported the development and promotion of key ASPIS outputs. It successfully organized the 3rd annual ASPIS Open Symposium in 11-12 September 2024 in Copenhagen, Denmark, creating a platform for interdisciplinary collaboration and dissemination of key achievements. It also represented

ASPIS with a poster at the annual Helsinki Chemical Forum (HCF) on 30-31 October 2024 in Helsinki, Finland and the European Partnership for Alternative Approaches to Animal Testing (EPAA) Annual Conference on 13 November 2024, Brussels, Belgium. The C&DWG supported the mentoring programs under the ASPIS Academy, empowering early-stage researchers (ESRs) and fostering the next generation of NAM experts.

Status

The C&DWG will continue its activities.

Computational Approaches Working Group (CAWG)

Co-Chairs: Emilio Benfenati (ONTOX), Gerhard Ecker (RISK-HUNT3R), and Nate Keith (PrecisionTox)

Background

The Computational Approaches Working Group (CAWG) is responsible for coordinating activities related to computational methods within ASPIS. These activities encompass: (a) the development of in silico models and tools for relevant property evaluation, (b) methodological enhancements to improve computational techniques that can be shared across ASPIS projects, and (c) supporting other WGs with predictive models and expertise. In 2024, CAWG efforts were directed toward data sharing, model development, and collaborative work with ASPIS WGs.

Successes and Opportunities

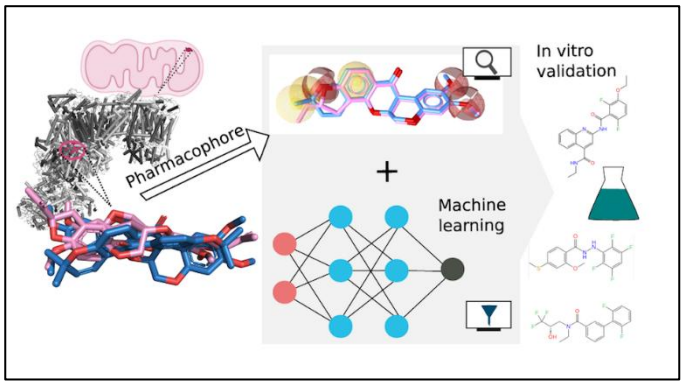

Figure 8. Structure-based Pharmacophore Models

A shared repository for chemical datasets and models from partners was established to facilitate data exchange and modelling efforts, supported by collaborations across ASPIS. Collaborative activities with the Risk Assessment WG were set-up to provide computational predictions for the cyproconazole case study to enhancing hazard assessment processes (Figure 8). Similar collaborations are foreseen for other endpoints and case studies. Tools like Namastox and OpenModel were proposed for documenting and deploying models effectively.

Shortcomings and Uncertainties

The integration of diverse tools continues to pose challenges. Differences in model structures, protocols, and endpoints require detailed analysis to ensure compatibility and meaningful integration. Moreover, some models are proprietary and cannot be shared directly. Coordination with other WGs requires clearer articulation of needs and better feedback mechanisms.

Aims for 2025

CAWG aims to explore further methods for combining predictions from individual models to improve the accuracy and reliability and to align efforts across the consortium. To better associate its activities with practical cases, and to contribute to the implementation of ASPA and OPRA, i.e. to verify the utility of the models developed within the perspective of risk assessment, the proposal is that the activities of the CAWG will stop as separate ones, and the group will merge within this overarching, more ambitious perspective.

Impact

The availability of the new models, freely available, with high performance, will facilitate the screening of chemical substances, to be done by the different stakeholders, including industry and regulators. The in silico tools will improve the possibility of assessing the safety of substances too. These in silico tools will be easily integrated into the new architecture, under implementation, addressing the series of assays and tools for risk assessment.

Status

The CAWG will discontinue its activities and participate in the ASPA Task Force.

Database Working Group (DbWG)

Co-chairs: Barry Hardy (RISK-HUNT*3R*), Tomasz Ignasiak (PrecisionTox) and Thomas Luechtefeld (ONTOX)

Background

The ASPIS Data Working Group was formed in early 2023 to support collaboration concerning data-driven resources supporting ASPIS goals. The group had several initial meetings to establish the group and discussing different activities of group members.

Success and Opportunities

A focus goal that the group has established is to work together to develop knowledge resources supporting ASPIS case study goals starting with the steatosis case study. Following up on the work of the ASPIS Compound Selection Working Group, we have assembled several inputs on compound lists, including selection work on ONTOX, network modelling on RISK-HUNT*3R*, inputs from the EC JRC and public resources. We are building a knowledge graph to represent current knowledge of the steatosis network and the relationships between key events and the adverse outcome. This has in turn raised the issue of needing a common ontology for the terms describing nodes and relationships in the steatosis network model, which is currently a work in progress.

During 2024, we progressed the work initiated in 2023 with the following activities:

- We released the ASPIS compound database containing all relevant compounds and associated properties selected for the ASPIS projects (Figure 9). This database is now available to all project members as an open access science resource. Ongoing work to increase impact includes the ability to bring in further properties through automated workflows and the annotation of compounds for selection criteria and mechanistic evidence.
- We completed and released a knowledge graph based on EU-ToxRisk data, the forerunner EU project to ASPIS. Further work included the establishment of workflows to connect compound-related data to analysis workflows including providing pathway analysis and points of departure for risk assessment such as documented in the ASPA workflows within the Namastox application.

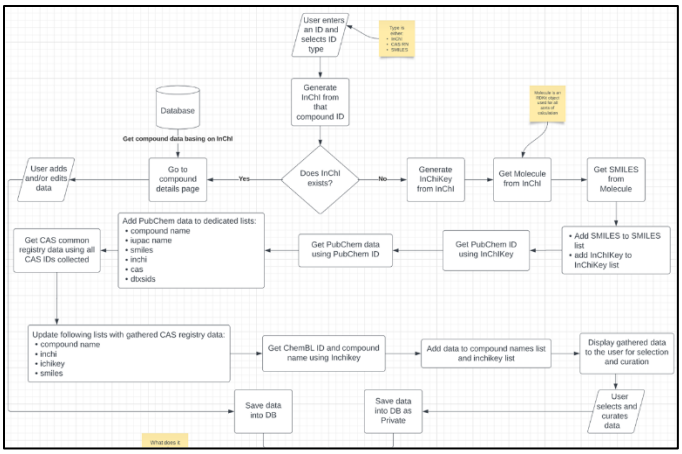

Figure 9. Compound Database Properties Workflow

- We developed the ontology for an initial steatosis network model and implemented it into a knowledge graph. We developed a framework for inclusion of different network models including ones based on systematic review or MIE hypothesis. This work will be ongoing in 2025 supporting the inclusion of computational and experimental evidence for hypothesis testing and model comparisons.
- We completed integration work combining the compound database, knowledge graphs and biological data (e.g., omics response signatures) supporting case study work.
- We initiated work using AI methodology applied to the databases and knowledge graphs, which will be ongoing work in 2025.

Shortcomings and Uncertainties

Development of the data and knowledge resources is a substantial goal, so we will need to sustain motivation and provide credit for active contributors. This can be helped by dissemination activities include presentation opportunities for younger scientists in the group and the opportunity to participate in a couple of high impact joint peer-reviewed publications. An additional challenge we will face is the capability to support adequately users as we expand resource access. We will need to align this activity with sustainability measures at both the project and cluster level, including contributing to the new ASPIS Task Force activities planned for 2025.

Aims for 2025

In 2025, our plan is to refine and complete the deliverables related to the above activities and to disseminate and support their value propositions, including both scientific and sustainability impact goals. We plan to release, present and discuss these resources at conference events during 2025 including SOT, SETAC, WC13, EUROTOX and the ASPIS Open Symposium.

Dissemination Activities

In 2024 we presented our progress at several meetings including working group meetings, EUROTOX, the ASPIS Open Symposium, the FDA-organised GSRS regulatory science conference, the ECETOC workshop on AI in Risk Assessment, and the OpenTox 2024 virtual conference which included several presentations related to this work, including articles in preparation for *In vitro* Toxicology.

Impact

The impacts of the DbWG include making available the data and knowledge on compounds and mechanisms supporting ongoing case studies and publications from ASPIS project members. It is also providing broader access to a NAMs knowledge base to non-ASPIS researchers in the field. The DbWG is contributing to ongoing NAMs research and risk assessment activities through the establishment of sustainable open access scientific resources, supporting reproducible science and regulatory acceptance goals through the provision of well-documented evidence with high data integrity. Finally, it supports the development of new NGRA best practices by connecting data and knowledge to ASPA workflows.

Status

The DbWG will merge with the CSWG and continue its activities.

Kinetics & Exposure Working Group (KEWG)

Co-chairs: Sylvia Escher (RISK-HUNT*3R*), Beate Escher (PrecisionTox) and Nynke Kramer (ONTOX)

Background

The Kinetics & Exposure Working Group (KEWG) consists of investigators from each of the three consortia that work on defining chemical exposure levels in the environment, human populations, target organs and *in vitro* assays. Complementary NAMs, namely (1) aggregated exposure assessment tools, (2) physiologically based kinetic modelling and (3) *in vitro* distribution kinetics models, are being developed in each of the three consortia that will benefit from being integrated into a common, pragmatic guideline for risk assessors to perform quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) and NGRA. The aim of this working group is to publish on the ASPIS website a guideline detailing a tiered approach to exposure and kinetics assessment using the tools used and developed in each of the three consortia and illustrate the approach with ASPIS case studies by September 1, 2026, when the individual projects will have ended.

Successes and Opportunities

Regular online meetings with consortia members with an interest in the subject matter are organised monthly and chaired by one of the three co-chairs. During each meeting, an update of the work done by individual partners on the ASPIS case study is given.

Overarching activity for ASPIS in 2024 is a case study on steatosis. Within this working group, the triazole fungicides were chosen as case study chemicals. Exposure to these chemicals is associated with liver steatosis in humans, they are included in the chemical lists of the three consortia and there is internal and external exposure data available for this group. The case study should allow the working group to define the applicability domain of and assign weights-of-evidence to individual exposure assessment tools used and developed in the three consortia.

The case study has finished several steps including the 1) collection of physiochemical and toxicokinetic ADME (absorption, distribution, metabolism, excretion) values for a selection of triazoles to compare experimental, in silico and *in vitro* derived values; 2) a comparison of free and cell-associated triazole concentrations in *in vitro* ADME and steatosis systems estimated by *in vitro* distribution kinetics models (Figure 10). For 2024, the aim is to include the model estimates of the Fischer et al. (2017) (DOI:

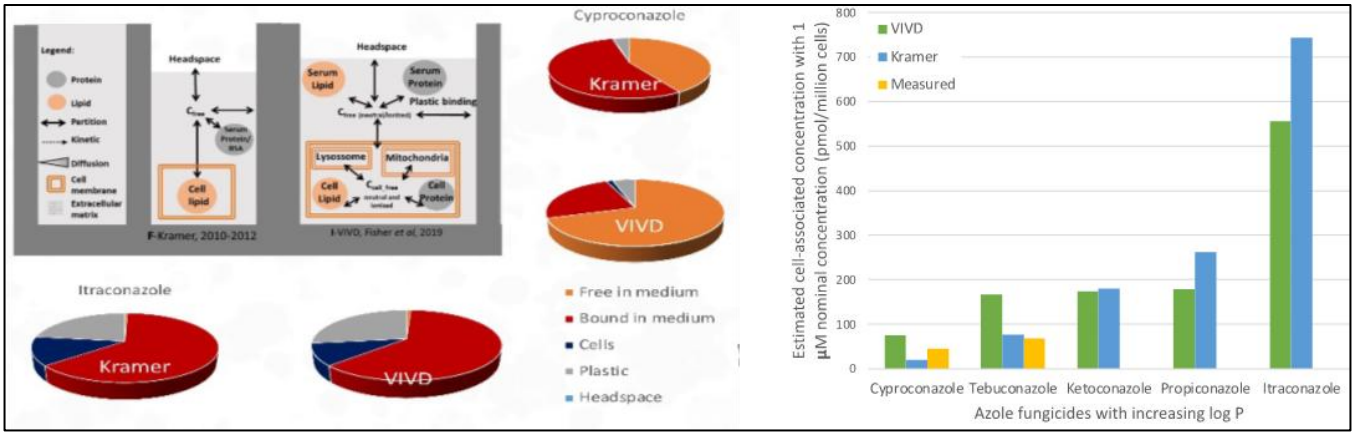

Figure 10. Comparison of free exposure medium concentrations in a steatosis screening assay.

Comparisons were made using the Kramer (ONTOX) and VIVD (RISK-HUNT*3R*) in vitro distribution kinetics models. ONTOX measured cell-associated concentrations in the HepaRG steatosis assay to evaluate the model predictions of cyproconazole and teubuconazole.

10.1021/acs.chemrestox.7b00023) model, which is used in PrecisionTox. Currently, the levels in medium, plastic and cells of a selection of six triazole chemicals in the ONTOX steatosis assay with HepaRG are being assessed. The plan is to finalise the experiments soon to compare experimental with model estimates. As a deliverable, a manuscript for publication is planned for June 2025. The human oral dosing regimens and plasma and liver concentrations of triazole fungicide exposure associated with steatosis using PBK modelling were predicted. *In vitro* intrinsic clearance assays using primary human hepatocytes (RISK-HUNT3R) and HepaRG (ONTOX) were measured and integrated as compounds spcfifc data into the PBK modelling. PBK model estimated of maximum plasma concentrations and repeated external (oral) doses in rats and humans using the different models such as PK-Sim and Simcyp™.

The Fischer model was applied to predict freely dissolved concentrations and fraction freely dissolved f_{free}. Using the following equation, derivation /prediction of the input physicochemical parameters $D_{BSA/w}$,as a proxy for protein binding and $D_{lip/w}$, as a proxy for lipid membrane binding, were refined.

$$f_{free} = \frac{1}{1 + D_{BSA/w} \times VF_{protein,medium} + D_{lip/w} \times VF_{lipid,medium}}$$

The affinity to biological membranes expressed as $D_{lip/w}$ ranged from 1.6 to 5.8 thereby covering four orders of magnitude. Affinity to albumin and structural proteins was smaller but encompassed several orders of magnitude (Figure 11A).

The acidity constants of Itraconazole and Omeprazole were measured. Itraconazole is present at 3.52% in its neutral form at pH 7.4, Omeprazole 3.91% negative and 27.1% positively charged, the rest is neutral. Most other conazoles are predicted to be neutral or near neutral (Figure 11A).

In the exposure model, the number and lipid/and protein content of the cells did not play any role and in medium supplemented with 10% FBS, the binding to plate material was also negligible. Given the large range in hydrophobicity of the conazoles, the fraction freely dissolved in the bioassay medium varied largely from almost completely dissolved (89% for omeprazole) to almost completely protein bound (f_{free} 0.76% for itraconazole) (Figure 11B).

Shortcomings and Uncertainties

As with the previous report, similar shortcomings and uncertainties apply for the work within the working group of Kinetics and Exposure. There are time and resource constraints among members of the

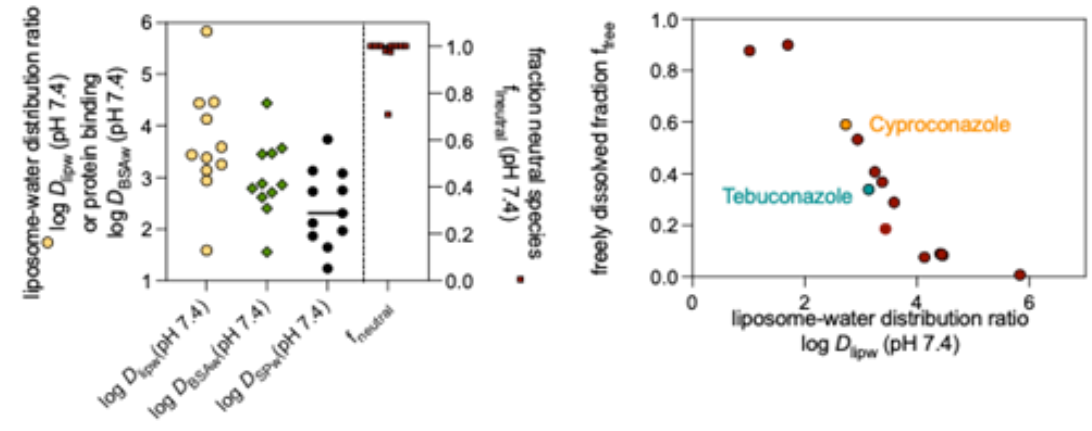

Figure 11. Physicochemical properties of the conazoles
(A) Physicochemical properties and fraction of neutral species of the conazoles.
(B) Freely dissolved fraction in a 384-well plate bioassay with a medium containing 10% FBS

KEWG due to the voluntary structure of the WGs. Exposure assessments are prohibitively laborious and performing exposure assessments for all new, ASPIS-specific chemicals is therefore infeasible. It is uncertain if sufficient exposure and kinetic information will be available to quantify uncertainty and variability of in vitro and in silico ADME tools.

Aims for 2025

As already outlined above, RISK-HUNT*3R* prepared a tiered testing and assessment approach for the testing and assessment of the kinetic parameters of compounds to enable *in vitro* to *in vivo* extrapolation approaches. In 2025, we will map the learnings from the above-described case study to this proposal to further mature the criteria and assessment elements.

Dissemination activities

- Poster presentation at the EUROTOX 2024 in Copenhagen, Denmark, 8–11 September 2024, presented by Sylvia Adam (ONTOX) on “Distribution Kinetics of Azole Fungicides in an *In Vitro* Liver Steatosis Model”.

Impact

The KEWG is contributing to advance the integration of NAMs predicting the (toxico)kinetics and assessing exposure to chemicals into NGRA. It does so by including nodules on kinetics and exposure, and associated tools, to ASPA NGRA workflow. It tests the workflow using the case study on the steatotic potential of azole fungicides. In so doing, KEWG ensures its work's translational value, contributing to the future adoption of ADME related NAMs in chemical safety assessment.

Status

The KEWG will merge with the RAWG in leading the ASPA Task Force.

Omics Working Group (OWG)

Co-chairs: Florian Caiment (ONTOX), Giulia Callegaro (RISK-HUNT*3R*) and John Colbourne (PrecisionTox)

Background

The ASPIS Omics Working Group (OWG) has been dedicated to advancing the integration of omics technologies into NGRA. Comprising experts from the ONTOX, PrecisionTox and RISK-HUNT3R consortia, the OWG convened monthly meetings throughout 2024 to discuss state-of-the-art data analysis strategies, present relevant software tools and share statistical best practices. Each member could bring forth specific omics-related challenges to benefit from the collective expertise of the group, thereby promoting robust and reproducible approaches to omics data interpretation. The OWG also contributed directly to the ASPIS overarching case study on steatosis, ensuring that any omics datasets selected by other WGs were processed and analysed using best practices.

Successes and Opportunities

In support of the ASPIS steatosis case study, we compiled a core dataset of over 1,200 microarrays. These data, sourced from public repositories; including TG-Gates, DrugMatrix and TransQST; were assembled, normalized and subjected to rigorous quality control before being distributed to OWG members (Figure 12). The CSWG provided a curated list of steatotic and non-steatotic chemicals, which guided our data selection. The overarching question, "Can heterogeneous transcriptomics datasets effectively identify the steatosis state of a compound?",inspired the formation of sub-teams within the

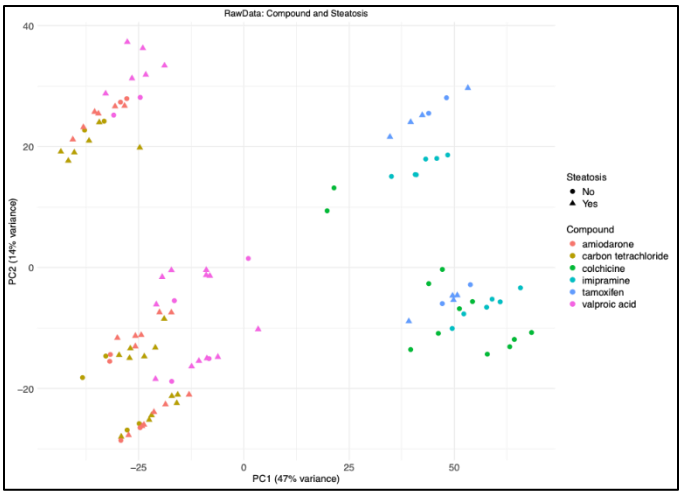

Figure 12. PCA plot showing steatotic and non- steatotic chemicals and their cognate chemical class

OWG, each tasked with applying distinct analytical strategies. Additional ongoing analyses leverage metabolomics datasets to refine further our understanding of steatosis-related pathways.

Our efforts have also included close collaboration with the ASPA WG to ensure that omics-derived insights fit seamlessly into the broader ASPIS framework. Beyond the internal synergy, we are finalizing several manuscripts that highlight the utility of omics data and machine learning approaches for chemical risk assessment:

- **Machine Learning Classification of Steatogenic Compounds Using Toxicogenomics Profiles:**
  This manuscript demonstrates the predictive power of machine learning algorithms, particularly Support Vector Machines (SVM), in distinguishing steatogenic from non-steatogenic compounds using human and rat transcriptomic data. SVM models reached high ROC-AUC values (0.82 for primary human hepatocytes and 0.96 for rat data), underscoring the potential of *in vitro* omics assays to anticipate *in vivo* outcomes.
- **Steatosis Case Study DEG Analysis:**
  This manuscript focuses on a differential expression (DE) analysis approach to identify transcripts associated with steatotic states. Through a series of comparisons—pooled steatotic compounds versus controls at various doses—we uncovered a set of DEGs consistently modulated across exposure levels. Pathway analyses implicate lipid metabolism, lipoprotein assembly and immune-related functions, strengthening the biological plausibility of these candidate biomarkers and enhancing our mechanistic understanding of drug-induced steatosis.
- **Metabolomics-Based Identification of Biomarkers for Predicting Drug-Induced Steatosis:**
  Complementary to the transcriptomics studies, this manuscript describes metabolomics analyses that distinguish steatotic from non-steatotic compounds based on characteristic metabolic signatures. Identified metabolites (e.g., specific amino acids, lipids, betaine and spermine) connect to key metabolic pathways (glutathione, phosphatidyl biosynthesis and carnitine metabolism), offering candidate metabolite biomarkers. Future integrative analyses with transcriptomic data aim to confirm and refine these predictive biomarkers.

Shortcomings and Uncertainties

While the data collected for the steatosis case study proved sufficient to produce these three manuscripts, the process highlighted ongoing challenges in data acquisition. Securing similarly comprehensive datasets for another complex toxicity endpoint would be difficult. The heterogeneity of public data sources, as well as the dependence on historical studies with varying quality standards, remain hurdles in ensuring robust, reproducible analyses for NGRA applications.

Aims for 2025

- As of November 2024, the OWG has officially dissolved. Its members are now encouraged to join one or more of three newly formed ASPIS taskforces, each designed to advance specific strategic goals:
  - *Impact*: Maximizing the real-world applicability of ASPIS outputs in regulatory and industrial contexts.

- *Sustainability*: Ensuring the long-term maintenance of computational tools, data repositories and best-practice guidelines.
- *Risk Assessment Initiatives*: Operationalizing omics-based models and biomarkers into standardized, animal-free chemical safety evaluation frameworks, developed under ASPA and OPRA.

Dissemination Activities

- ASPIS training June 2024 "Omics data analytics" Giulia Callegaro (RISK-HUNT3*R*) & Florian Caiment (ONTOX)
- JRC-EPAA Designathon: 10 different proposals from member of the ASPIS cluster.
- SOT 2025 talk: "Omics Data and Regulatory Frameworks: Success and Challenges", Florian Caiment (ONTOX)
- JRC Summer School on Non-Animal Approaches in Science, May 2025, Florian Caiment (ONTOX)

Impact

The OWG is contributing to advance the integration of high-throughput omics technologies into NGRA. By leveraging its multidisciplinary expertise, the Omics group has demonstrated how omics datasets, including transcriptomics and metabolomics, can uncover mechanisms of chemical toxicity and identify biomarkers with high predictive power. The group's contributions to the ASPIS steatosis case study have not only refined the understanding of steatosis-related pathways but also showcased the utility of machine learning in deriving actionable insights from heterogeneous data. These advancements directly support the 3R's principles, particularly focusing on replacing traditional animal-based approaches, and highlight the potential for omics-derived methodologies to meet regulatory standards. By aligning its outputs with ASPA and broader ASPIS frameworks, the OWG ensures its work's translational value, contributing to the future adoption of innovative, non-animal approaches in chemical safety assessment.

Status

The OWG will discontinue its activities and participate in the ASPA Task Force.

Quantitative Adverse Outcome Pathway Working Group (qAOPWG)

Co-chairs: Anouk Verhoeven (ONTOX), Pu Xia (PrecisionTox) and Mark Cronin (RISK-HUNT3*R*)

Background

The scope of the quantitative Adverse Outcome Pathway Working Group (qAOPWG) is to support the development of qAOPs across ASPIS. Specifically, this WG aims to investigate models that quantify Molecular Initiating Events (MIEs) or Key Event Relationships (KERs) within existing AOPs using non-confidential data, as well as identifying and sharing good practice. Specific activities involve bringing benefit to qAOP development across ASPIS by developing common ideas. This would include jointly finding solutions to problems and being able to share knowledge of dose-responses, data and models across the three projects. The qAOPWG aims is to develop one or more common qAOPs, including those from linear and network AOPs that are of interest to all partners, such that data and expertise can be combined. The WG will facilitate integration of qAOPs with MIE and PBPK modelling to enable Quantitative Systems Toxicology approaches (Figure 13). In terms of application, the qAOPWG will identify how risk assessors could apply qAOPs, with an emphasis on regulatory use. As part of

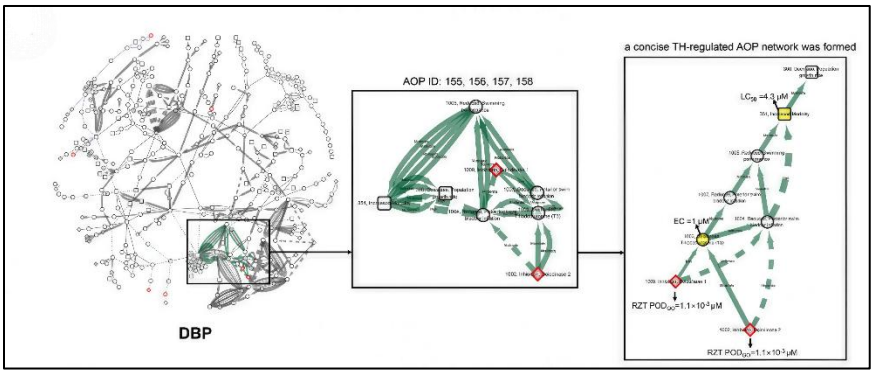

Figure 13. Quantify the qAOP relationships: Omics, *in vitro* and *in vivo*

regulatory use, the degree of confidence that risk assessors need to use qAOPs in risk assessment will be considered, along with obstacles/ concerns for assessors to use qAOPs in risk assessment.

Successes and Opportunities

In 2024, the qAOPWG established three sub-working groups (sub-WGs): the validation qAOP sub-WG, the steatosis qAOP sub-WG and the developmental neurotoxicity (DNT) sub-WG. The primary activities are concentrated in the first two sub-WGs. The steatosis qAOP sub-WG focuses on applying dose- and response-response modelling to quantify KERs within the AOP network for chemical-induced liver steatosis. To initiate this work, the KERs with the highest confidence levels, supported by the most consistent data, are prioritized. The validation qAOP sub-WG is establishing a framework to assess the validity of qAOPs. A paper titled, *A Framework to Evaluate, Verify and Assess the Validity of quantitative Adverse Outcome Pathways (qAOPs)* has been assembled and tasks for completion have been distributed and will be completed at the beginning of 2025.

Shortcomings and Uncertainties

There has been a limited critical mass of members with sufficient expertise in the different types of qAOP models, especially regarding the DNT qAOP. Further, the position and future application of qAOPs with ASPA NGRA framework requires a further vision. Therefore, the qAOPWG will transition into the ASPA Task Force.

Aims for 2025

The qAOP WG will be discontinued as the ASPIS concept shifts its focus to the three Task Forces: Impact, Sustainability and ASPA. In this context, the steatosis qAOP sub-WG will transition to the ASPA task Force, aiming to quantify the most supported KERs within the AOP network for liver steatosis, which will ultimately be integrated into the ASPA framework. Meanwhile, the validation qAOP sub-WG will contribute to the Sustainability Task. A paper on the validity and regulatory application of qAOPs will be completed in the first half of 2025. This framework is designed to enhance understanding, build confidence in qAOPs and support their application in chemical risk assessment.

Dissemination Activities

- Oral presentations at the Physicians Committee for Responsible Medicine “2024 Summer Immersion on Innovative Approaches in Science”, Washington DC, USA, 31 May 2024.
- Oral presentations in the session “New Approach Methodology and Kinetic Modeling Approaches to Support Read-Across” at the 63rd Annual Meeting of the Society of Toxicology (SOT), Salt Lake City UT, USA, March 2024.
- Oral and presentations at the Bundesinstitut für Risikobewertung (BfR – German Federal Institute for Risk Assessment), Berlin, Germany, June 2024.Oral presentation in the session “New Approach Methodology in Ecotoxicology and Risk Assessment - Theory and Application” at SETAC Asia-Pacific 14th Biennial Meeting, Tianjing, China, September 2024.

Impact

The qAOP WG has provided a forum for the discussion of a range of qAOP approaches. qAOP model development will allow for the implementation of NAMs to predict relevant key events and adverse

outcomes. Thus, data from *in vitro* NAMs can be extrapolated, and then made usable and implementable for hazard identification, supporting animal-free chemical safety assessment. These qAOPs are being developed as part of tiered assessment strategies, notably the ASPA and will support the practical implementation of those strategies. The qAOP WG is also working to develop an approach to evaluate and validate qAOPs. This will impact their applicability for regulatory purposes and is intended to promote and encourage their use and acceptance.

Status

The qAOPWG will discontinue its activities and participate in the ASPA Task Force.

Risk Assessment Working Group (RAWG)

Co-chairs: Mirjam Luijten (RISK-HUNT3R), Erwin L. Roggen (ONTOX) and Stefan Scholz (PrecisionTox)

Background

The three ASPIS consortia have complementary approaches on how to use NAMs for the hazard and risk assessment of chemicals including prioritization, grouping/read-across and hazard characterization. The ASPIS Risk Assessment Working Group (RAWG) intends to share and link different approaches, coordinate joint activities and critically review ASPIS research in comparison to previous activities for promoting NAMs with reference to the EU-ToxRisk project. The RAWG also aims to identify gaps, limitations and advantages of chosen approaches. It will compare approaches and results to those outside of ASPIS, particularly with a global view to identify targets for hazard and risk assessment to plan for joint/coordinated activities. Furthermore, it aims to connect research activities in ASPIS to form joint case studies. Together with other WGs, it will facilitate the requirements for hazard and risk assessment by end-users and stakeholders. Finally, the RAWG ensures complementarity of its activities with PARC to support the European Union and national chemical risk assessment and risk management bodies with new data, knowledge, methods, networks and skills. This will facilitate the transition to next generation evidence-based chemical risk assessment.

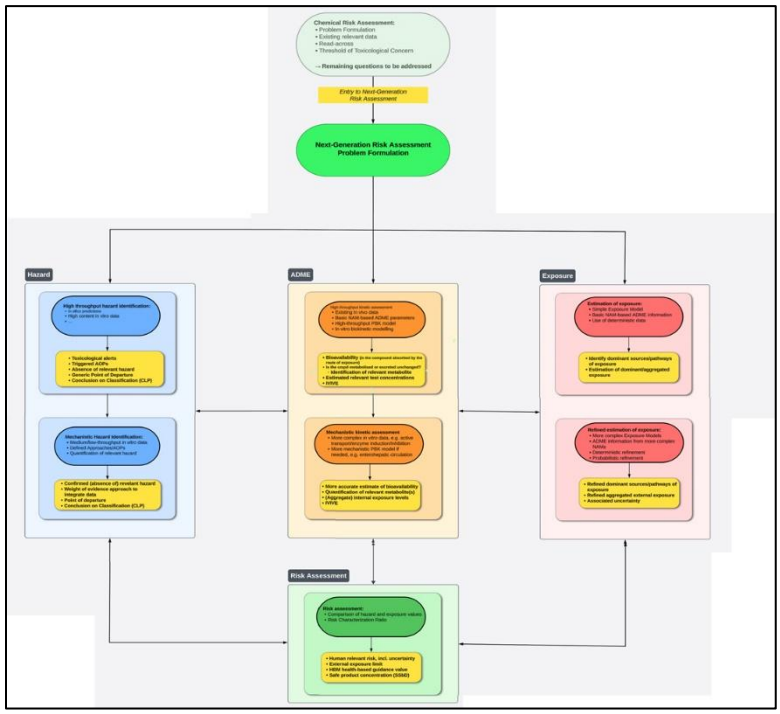

Figure 14. Generic outline of the ASPA workflow

Successes and Opportunities

The RAWG established regularly scheduled inter-project meetings. They supported discussions, development and presentations of ASPA (Figure 14). A highlight was the presentation and discussion of ASPA with members of the OECD Expert Group that aims to develop a framework for NGRA approaches for systemic toxicity in the context of the OECD Hazard Assessment Programme. With the ongoing development of ASPA,

the RAWG has particularly strengthened its interactions with the Kinetics and Exposure WGs to develop, design and collect information for a case study with conazoles. This case study is ongoing.

Shortcomings and Uncertainties

It is unclear yet how exactly the RAWG will interact with the other WGs and increase its engagement with regulatory bodies. This could be resolved by establishing a realistic annual plan with milestones and timelines. Particularly the testing and improving of ASPA will require the interaction with other WG, to provide information such as exposure or omics responses for demonstration examples.

How Probabilistic Risk Assessment (the ONTOX flagship) and results from having evolutionary knowledge of the mechanisms of toxicity (a key PrecisionTox contribution) will be incorporated remains unclear. In 2024, the first case studies involving Probabilistic Risk Assessment will be performed by ONTOX in collaboration with stakeholders from industry and regulatory environment. It is anticipated that these case studies, some involving ASPIS chemicals and fitting into the ASPIS case study repertoire, others involving chemicals of interest for industry stakeholders and stakeholders from the regulatory environment.

Aims for 2025

In 2025, the RAWG we will evaluate the ASPA workflow against further ASPIS case studies.

Dissemination activities

- ASPIS OS, Copenhagen, September 2024: Presentation on the RA activities.
- Joint interview of ASPIS chairs Mirjam Luijten and Stefan Scholz, prepared by ALTERTOX: *www.youtube.com/watch?v=QBEvPxh4igQ*
- OECD Expert Group on NGRA for systemic toxicity; 12 November 2024, online
- *NURA Dynamic Discussion*; 14 November 2024, webinar
- OECD IATA case studies project; 19 November 2024, online

Impact

We expect a significant impact by the conduction of the case study with conazoles and by comparison of ASPA with the results of present assessments based on animal testing. We intend to achieve a similar risk assessment with similar protection levels using ASPA. Results of the case study will be used to improve ASPA and to design further case studies.

Status

The RAWG will merge with the KEWG in leading the ASPA Task Force.

ASPIS PUBLICATIONS (NOVEMBER 2023 TO NOVEMBER 2024)

ONTOX (No. 963845)

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