Concept Article

An Alternative Safety Profiling Algorithm (ASPA) to Transform Next Generation Risk Assessment into a Structured and Transparent Process

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Abstract

Next generation risk assessment (NGRA) strategies use animal-free new approach methodologies (NAMs) to generate information concerning chemical hazard, toxicokinetics (ADME), and exposure. The information from these major pillars of data gathering is used to inform risk assessment and classification decisions. While the required types of data are widely agreed upon, the processes for data collection, integration and reporting, as well as several decisions on the depth and granularity of required data, are poorly standardized. Here, we present the Alternative Safety Profiling Algorithm (ASPA), a broad-purpose, transparent, and reproducible risk assessment workflow that allows documentation and integration of all types of information required for NGRA. ASPA aims to make safety assessments fully traceable for the recipient (e.g., a regulator), delineating which steps and decisions have led to the final outcome and why certain decisions were made. An overarching objective of ASPA is to ensure that identical data input yields identical outcomes in the hands of independent assessors. Therefore, ASPA is not just a data gathering workflow; it also considers data interdependencies and requires precise justification of intermediate decisions. This includes the monitoring and assessment of uncertainties. To assist users, the *ASPA-assist* software was developed. It formalizes the reporting process in a reproducible and standardized fashion. By guiding an operator step-by-step through the ASPA workflow, a complete and comprehensive report is assembled, whereby all data, methods, operator activities, and intermediate decisions are recorded. Practical examples illustrating the broader applicability of ASPA across various regulations and problem formulations are provided through case studies.

Plain language summary

Researchers and safety experts have developed animal-free test methods to assess chemicals. These include *in vitro* tests, computer models, and simulations of how a chemical behaves in the body. While the required information is clear, the way it is collected, combined and reported is not standardized. The Alternative Safety Profiling Algorithm (ASPA) provides a transparent, reliable and standardized workflow for chemical safety assessment based on non-animal methods. It records all steps and decisions, tracking uncertainties, showing how conclusions are reached and why certain decisions were made. The *ASPA-assist* software guides users step-by-step and assembles comprehensive reports. ASPA and *ASPA-assist* are presented and explained here. Case studies are used to show how ASPA can be applied across chemicals and regulations.

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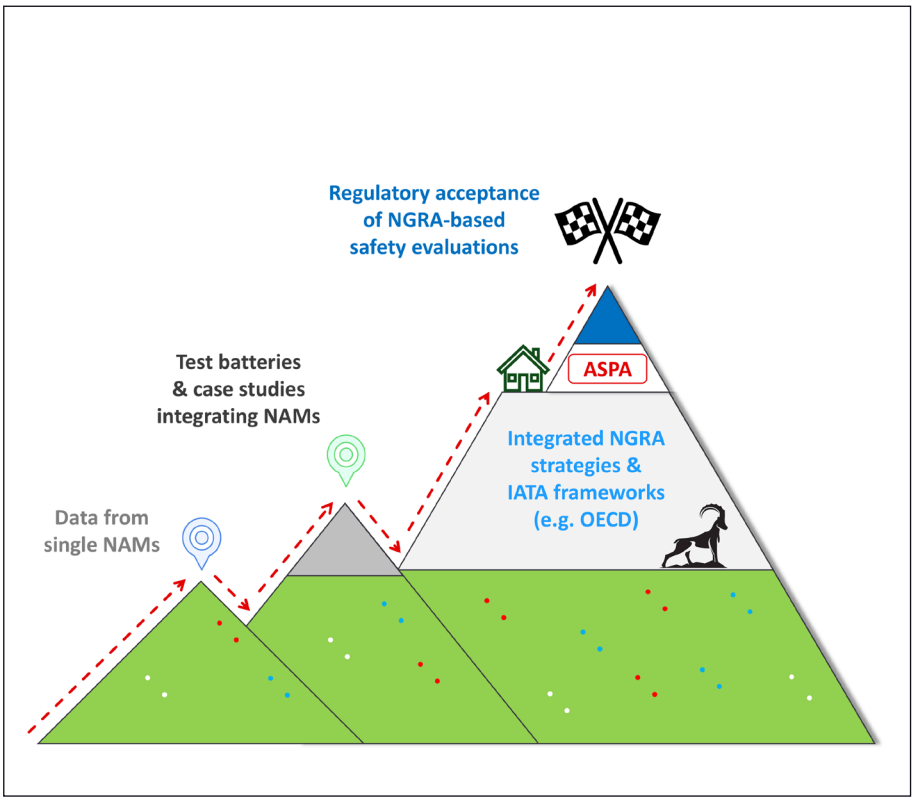

Fig. 1: Positioning of ASPA on the path from the use of single NAMs towards regulatory NGRA applications

For the evaluation of systemic toxicity, data from a single NAM can sometimes provide important, possibly necessary, information. However, this will usually not be sufficient. A further step towards NGRA is the use of batteries of NAMs. These typically produce complex, high-dimensional data that need integration. How this may be done has been demonstrated by various case studies (see Tab. 1). Formal guidance on the data and information types required for NGRA has been given by the OECD in their IATA framework, and by various other initiatives. On the way towards a general regulatory acceptance of NGRA-based safety evaluations, more guidance on a formalized data integration and how to use these assembled data for risk assessment is still missing. ASPA is a tool designed to close this gap.

1 General background on NGRA#

The use of non-animal methods to assess the safety of chemicals requires the (i) generation, (ii) integration, and (iii) interpretation of complex sets of data. One important element is the use of a broad panel of animal-free new approach methodologies (NAMs) to identify bioactivities and characterize these concerning potency and toxicological relevance. Such NAMs include both experimental and computational approaches. Complementary to this, information on the toxicokinetic behavior of the test compound must be generated. A major tool for this is the development and application of physiologically-based kinetic (PBK) models and the parametrization of such models with data from specialized NAMs that model aspects of absorption, distribution, metabolism, and excretion (ADME). Generation and behavior of metabolites can play an important role in safety assessment. Similarly, special distribution phenomena, e.g., transporter-dependent accumulation in certain cell types or tissues, need to be accounted for in the overall risk assessment. Moreover, the information domains are interdependent. One example (amongst many) is that some approaches used for hazard characterization and toxicokinetic prediction require information on the external exposure situation and the modelling of various exposure scenarios. The ensemble of all these approaches needs to be integrated via an overarching strategy, with defined sub-routines, to enable next-generation risk assessment (NGRA) of chemicals. Several inroads toward NGRA have been outlined previously (Tab. 1), and it is expected that they may not only reduce the reliance on animals for human safety assessment, but also improve the relevance, efficiency, and/or speed of future chemical risk assessment (Schmeisser et al., 2023; Walder et al., 2025; Balls et al., 2024; Tralau et al., 2015).

The use of single (or few) NAM data to inform on some aspects of toxicity is presently the state of the art in certain toxicological domains (e.g., acute topical toxicity). However, achieving adequate coverage of more complex toxicological domains (e.g., systemic toxicity) in a regulatory context remains a significant challenge. Ultimately, complex multifactorial data streams will be required for NGRA (Pallocca et al., 2022), and it is likely that several intermediate steps are necessary on the way to achieving this goal (Fig. 1). A milestone on this path is the definition of integrated approaches to testing and assessment (IATA) by the OECD (OECD, 2020) and of related strategies in drug discovery and the pharmaceutical industry (Beken et al., 2016; Desprez et al., 2019; Freires et al., 2023; Marx et al., 2025; Beilmann et al., 2019). Such

Abbreviations: ADME, absorption, distribution, metabolism and excretion; the term is used here equivalent to "toxicokinetics"; AI, artificial intelligence; AOP, adverse outcome pathway; ASPA, alternative safety profiling algorithm; DNT, developmental neurotoxicity; DP, decision point; ECHA, European Chemicals Agency; EFSA, European Food Safety Authority; HED, human equivalent dose; IATA, integrated approaches to testing and assessment; NAM, new approach methodologies; NGRA, next generation risk assessment; OECD, Organisation for Economic Co-operation and Development; PBK, physiologically based kinetic; PoD, point of departure; QSAR, quantitative structure-activity relationship; TG, test guideline; TTC, threshold of toxicological concern; WF, workflow

Tab. 1: Exemplary NGRA strategies designed to assess systemic repeat dose toxicity

First author	Title (shortened)/content
Bajard et al., 2023	Application of AOPs to assist regulatory assessment of chemical risks – Case studies
Ball et al., 2022	A framework for chemical safety assessment incorporating NAMs within REACH
Baltazar et al., 2020	An NGRA case study for coumarin in cosmetic products
Baltazar et al., 2025	Making safety decisions for a sunscreen active ingredient using NGRA: Benzophenone-4 case study
Basketter et al., 2012	A roadmap for the development of alternative (non-animal) methods for systemic toxicity testing
Berggren et al., 2017	A workflow based on exposure considerations and non-animal methods
Berggren and Worth, 2023	Towards a future regulatory framework for chemicals in the EU – Chemicals 2.0
Blaauboer et al., 2012	The use of biomarkers of toxicity for integrating *in vitro* hazard estimates into risk assessment for humans
Blaauboer et al., 2016	Considering NAMs in strategies for safety assessment of foods and food ingredients
Cable et al., 2025	Advancing systemic toxicity risk assessment: Evaluation of a NAM-based toolbox approach
Dearfield et al., 2017	Next generation testing strategy for assessment of genomic damage: A conceptual framework and considerations
Dent et al., 2018	Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients
Dent et al., 2021	Paving the way for application of NGRA to safety decision-making for cosmetic ingredients
Doe et al., 2025	Framework for classifying chemicals for repeat dose toxicity using NAMs
Fentem, 2023	Safer chemicals and sustainable innovation need regulatory use of modern safety science, not more animal testing
Herzler et al., 2025b	PARC's role in the uptake of NAMs and next-generation risk assessment into regulatory practice
Herzler et al., 2025a	Status report on NGRA route
Leist et al., 2014	Consensus report on the future of animal-free systemic toxicity testing
Luijten et al., 2020	Utility of a next generation framework for assessment of genomic damage: A case study using the industrial chemical benzene
Luijten et al., 2022	Prioritization of chemicals in food for risk assessment by integrating exposure estimates and NAMs: An NGRA case study
Magurany et al., 2023	A pragmatic framework for the application of NAMs in One Health toxicological risk assessment
Middleton et al., 2022	Are non-animal systemic safety assessments protective? A toolbox and workflow
Pallocca et al., 2022	NGRA of chemicals – The RISK-HUNT*3R* project perspective
Pereira et al., 2022	REACHing for solutions: Essential revisions to the EU chemicals regulation to modernize safety assessment
PrecisionTox, 2023	The Precision Toxicology initiative
Reynolds et al., 2021	A hypothetical skin sensitisation NGRA for coumarin in cosmetic products
Thomas et al., 2019	The next generation blueprint of computational toxicology at the U.S. Environmental Protection Agency
van der Ven et al., 2020	A case study with triazole fungicides to explore practical application of next-generation hazard assessment methods for human health
Vinken et al., 2021	Safer chemicals using less animals: Kick-off of the European ONTOX project

approaches have evolved from a loose definition of IATA key elements to increasingly defined sets of rules and requirements (concerning, for instance, quality, documentation, procedures, and (meta)data). The OECD has launched a case study program to highlight aspects of IATAs, to encourage exemplary applications in NGRA, and to provide learning material to further optimize the IATA definition[1]. In parallel, the program builds stakeholder confidence and facilitates further scientific progress concerning NGRA. Despite clear progress in many areas concerning NAMs (Blum et al., 2025; Cöllen et al., 2024; Holzer et al., 2023), implementation of an overall strategy remains a challenge.

Reliance on case studies alone may not be sufficient to demonstrate that an approach is (i) broadly suitable for a large variety of chemicals, (ii) applicable to many problem formulations, (iii) providing sufficient certainty of the outcome(s), (iv) being fully transparent concerning all tools used, (v) transparently justifying

[1] https://www.oecd.org/en/topics/sub-issues/assessment-of-chemicals/integrated-approaches-to-testing-and-assessment.html

and recording all intermediate and final decision points within the IATA process, and (vi) ensuring reproducibility (i.e., producing similar outcomes when performed in different countries or by different evaluators). To address these limitations, the ASPIS cluster[2] of European Horizon 2020 research projects – comprising RISK-HUNT*3R*, ONTOX, and PrecisionTox – developed the Alternative Safety Profiling Algorithm (ASPA)[3]. ASPA is an adaptable[4] workflow co-developed by many scientists and improved stepwise by stakeholder input and application in defined case studies. It guides scientists and regulators through all phases of NGRA, from problem formulation to risk characterization. Here we present a first overview of the ASPA workflow, describing its principles, structural outline, and its operability via the software interface *ASPA-assist*. The suitability of ASPA for real-world applicability in regulatory science is addressed in extensive case studies (Tab. S1[5]).

2 Scientific rationale on which ASPA is based

ASPA is built on three fundamental assumptions. First, it is assumed that exposure to a compound is a major determinant of its risk. Compounds without any significant internal exposure are considered to have low risk, and compounds with high internal exposure[6] (possibly accumulating at certain sites in the body or showing a very long elimination half-life) are considered candidates for high risk (to be assessed within ASPA). The exposure, ADME, and hazard pillars provide increasing levels of detailed information on these issues.

Second, ASPA assumes that NAMs can capture all relevant bioactivities of a test compound[7], so that testing strategies can be designed such that no activity relevant for toxicity is missed (avoidance of false negatives). Moreover, ASPA does not need to identify every bioactivity to yield results that are protective for the human population (Zobl et al., 2024). It is considered sufficient to detect all of the most potent, toxicologically-relevant bioactivities. This means that there may be unidentified bioactivities (acceptable in the ASPA process), but none of them would be more potent than the one with the highest potency identified. This approach aligns with the so-called "protective" risk assessment method, which involves identifying the highest concentration, dose, or exposure level that does not result in an adverse effect (Pallocca et al., 2022; Leso et al., 2025; Schmeisser et al., 2023). This implies that the exact adverse effect may not always be predictable (e.g., liver toxicity versus kidney toxicity), but the highest level (in terms of dose or intake, or in terms of internal exposure) of non-adverse effects can be defined.

Third, it is assumed to be possible to link bioactivities derived from human-relevant NAMs to adverse effects at the level of the whole organism (human). This involves interpreting NAM-derived data against the backdrop of biological and toxicological knowledge (e.g., utilizing adverse outcome pathway (AOP) databases, ontology maps of human physiological function or aggregate information compiled by AI approaches from relevant databases). Therefore, the ASPA workflow was designed to follow up on bioactivities to provide a toxicological plausibility for their relevance. Whenever possible, a mechanistic rationale is provided on why a certain compound activity (like a disturbed or activated process at a certain exposure concentration) is assumed to be relevant for an adverse effect.

Beyond agreement on its overarching principles, the implementation of ASPA necessitates a practical and systematic approach to navigate and integrate heterogeneous datasets as well as adapt to various regulatory contexts. Therefore, ASPA follows a set of design principles that prioritize (i) sufficient flexibility to support a broad range of regulatory scenarios; (ii) a guidance structure that ensures consistent decisions (across chemical evaluations and case studies); (iii) data transparency, including provenance information and FAIR principles (Blum et al., 2025; Wilkinson et al., 2016); (iv) clear decision points and their underlying rationale; (v) assessment progress traceability, including the overall gain in knowledge and assessment decision(s) following (intermediate) data integration; (vi) workflow reproducibility, ensuring that identical data and problem formulations lead to similar conclusions, independent of the safety assessor involved. The latter feature is a major gap in many current NGRA approaches but will be key to achieving confidence in the scientific and regulatory robustness of NAM-based risk assessment and therefore promote its acceptance.

3 Overview of basic ASPA modules and principles

Many earlier publications (Tab. 1) have defined key elements (here termed modules) required for NGRA. ASPA adopts and uses this generally accepted wisdom. The overarching six modules (Fig. 2) are the three major *de novo* data generation and integration pillars for

(i) external exposure,
(ii) ADME and
(iii) hazard, in addition to
(iv) the problem formulation (including a weight-of-evidence evaluation of already available data),
(v) a workflow for read-across; and

[2] https://aspis-cluster.eu/

[3] "ASPA" is assembled from the concepts of (i) "alternative safety profiling" (ASP), i.e., an overarching approach to an animal-free risk assessment (in a very broad sense) and (ii) using an algorithmic (second "A") flow scheme, to not just collect data, but to make the process more traceable and reproducible.

[4] "Adaptable" is meant to express that ASPA (i) accommodates various problem formulations, (ii) offers decision points that can switch between different downstream paths, and (iii) contains modules that may be activated to a different extent or at various temporal sequences.

[5] doi:10.14573/altex.2509081s1

[6] Note that for some current assessments external exposure plays an important role (e.g., in the absence of sufficient ADME data or human biomonitoring (HBM) data).

[7] Provided that the test compound's chemical and physicochemical properties are compatible with the testing in typical NAMs.

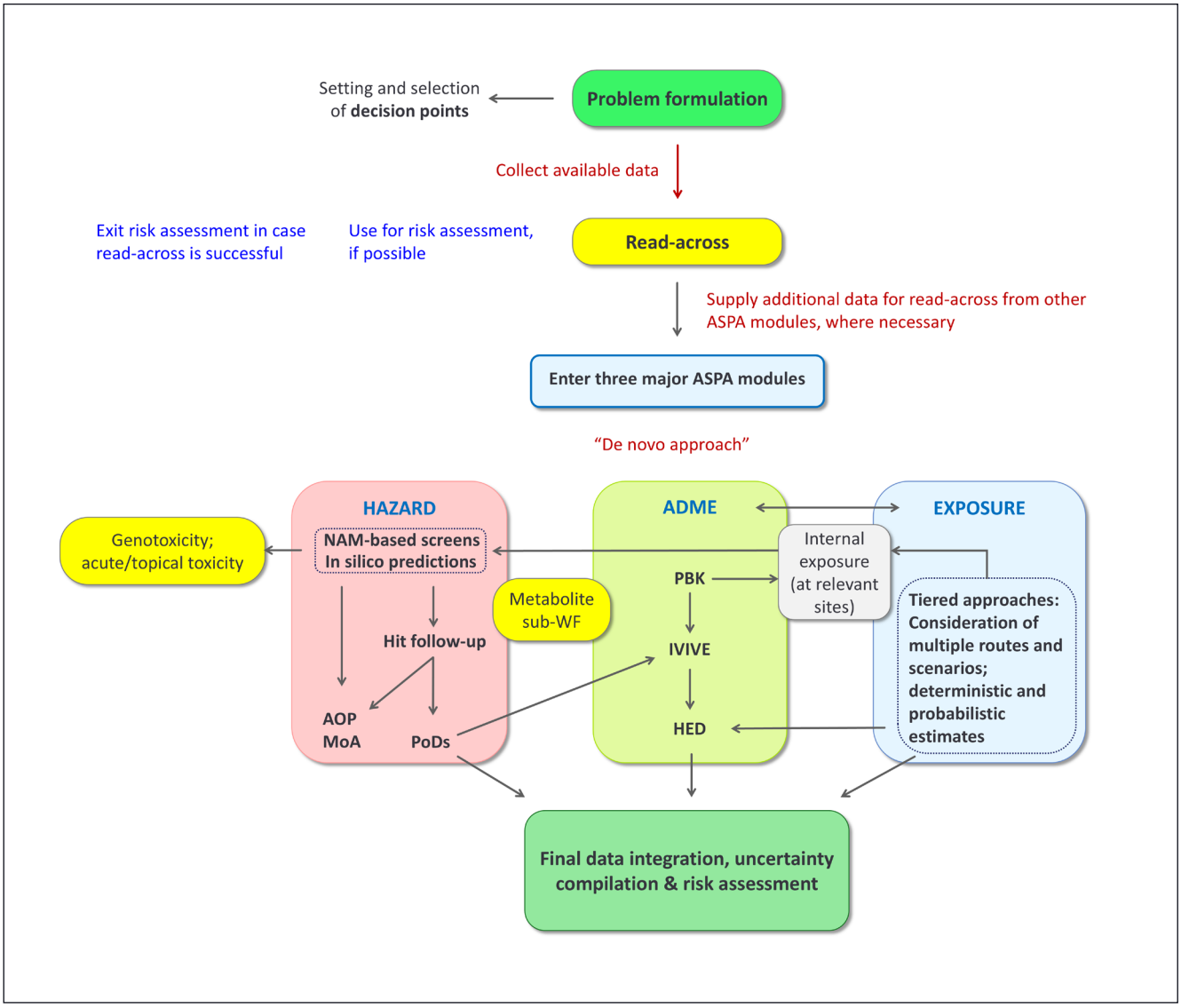

Fig. 2: Schematic overview of the ASPA construction

An overview of the overall ASPA construction is given in a simplified layout (applicable to all specific ASPA versions). Following the "problem formulation" and a "collection of available data", read-across is considered the first option (information in blue font informs on how this is incorporated in the algorithmic flow scheme). In case read-across is not possible or insufficient to address the problem formulation, the next option is a "*de novo*" approach. For this, data would be obtained in the three assessment pillars: hazard, ADME and exposure. Note that in practice, some of this work may occur in parallel, and different flows are possible (dependent on problem formulation and responses at decision points). Here one of the recommended sequences of documentation steps is displayed for exemplification of a potential (not mandatory) flow: Exposure information would inform the ADME pillar and allow the generation of data on expected internal exposures. This would inform on relevant test concentration ranges in the hazard module. A sub-workflow (sub-WF) on metabolites (formation and potential hazard) connects the hazard and ADME pillars.

A tiered approach in the hazard pillar leads from initial screening to a definite toxicity hypothesis (AOP/MoA) and a relevant NAM-based PoD that is converted by tools from the ADME pillar to a human equivalent dose (HED). This exemplary sequence does not exclude that an early exit point may be reached already after an initial hazard identification.

See Fig. S1[5] for a detailed view of ASPA 2.1. Different stages of data collection are indicated in red font; a specific graphical incorporation into the workflow was avoided to provide a simplified overview. MoA, mode-of-action; AOP, adverse outcome pathway; PoD, point of departure; IVIVE, *in-vitro*-to-*in-vivo* extrapolation; PBK, physiologically-based kinetics; ADME, toxicokinetics package considering absorption, distribution, metabolism and excretion.

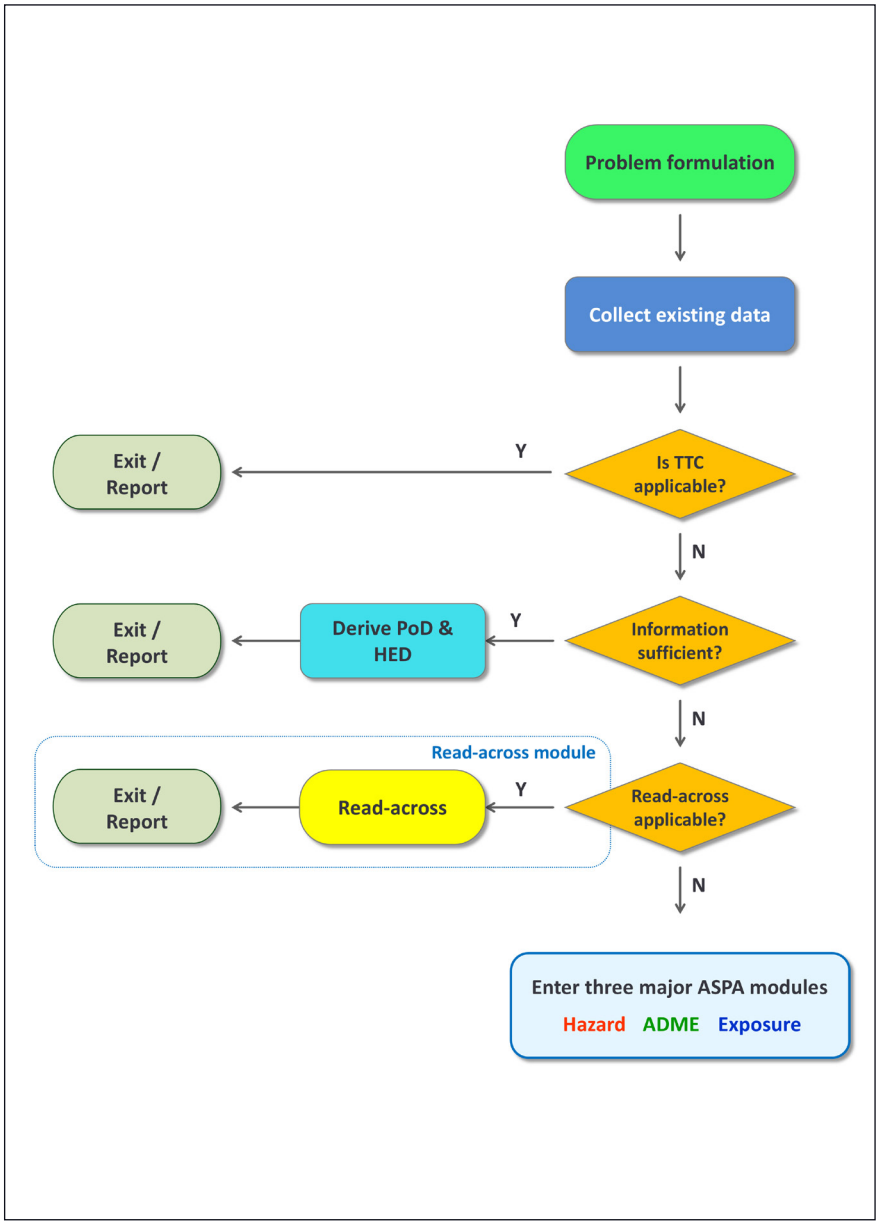

Fig. 3: Exemplification of an algorithmic structure and some of its elements
An exemplification of some structural elements (modules and building blocks) in the apical part of ASPA is given. Every ASPA case study starts with a "problem formulation" followed by a data generation building block to "collect existing data". Here, actual data together with metadata and the methods that have been used for their generation are collected and documented. This may include also exposure information. A first decision point (DP) would allow reporting and leaving of ASPA if the threshold of toxicological concern (TTC) principle is applicable and if TTC conditions are met. Note that this is a simplification of the actual ASPA structure, but it was adapted in this way to exemplify that ASPA has a strong focus on exposure. At a second decision point, available data may be considered sufficient for risk assessment or not. If yes, an operator task would require, e.g., deriving a human equivalent dose (HED), using an available physiologically-based kinetic (PBK) model. Another option is to use read-across (pursued in a specific module that is based on a detailed sub-workflow). In case this does not fully address the problem defined (or the outcome does not satisfy the data requirements), a "*de novo* risk assessment" on the basis of the main ASPA modules on exposure, hazard, and ADME (also named assessment pillars) is needed. *Note: this scheme shows general principles how decision points switch to different assessment options. The actual ASPA structure is more complex and differs in some details. It is presented in Fig. S1[5] (scalable file), as it is too detailed to be readable in a printed figure.*

(vi) an assembly of procedures related to the risk assessment and reporting.

More details are given below for the major pillars (i-iii) (Fig. 3).

The data generation and integration pillars all have a multi-tiered structure and interact with one another at several levels. This ensures that toxicological information becomes more refined and less uncertain, as the assessment progresses from tier to tier. Data integration does not only occur within one pillar but also across the pillars. Intermediate decision points within the ASPA workflow allow for a focus on or neglect of certain aspects. The decision processes are not only data-driven but also depend on the problem formulation. Moreover, the body of information required for an ASPA-guided assessment will be determined by the problem formulation and can vary among, e.g., a classification and labelling problem (EC, 2008), a full risk assessment of a plant protection product (EC, 2009), or many non-regulatory safety evaluations, such as a preliminary hazard characterization of a potential contaminant in a production process.

In this context, it is important to note that the ASPA workflow does not prescribe the specific approaches used to generate the required information. While it provides examples of suitable NAMs and guidance on how to report the resulting data, the workflow itself remains largely technology-agnostic. ASPA focuses on defining the type, quality, certainty, and granularity of information that should be produced at each step, while leaving the selection of specific NAMs or alternative approaches to the user.

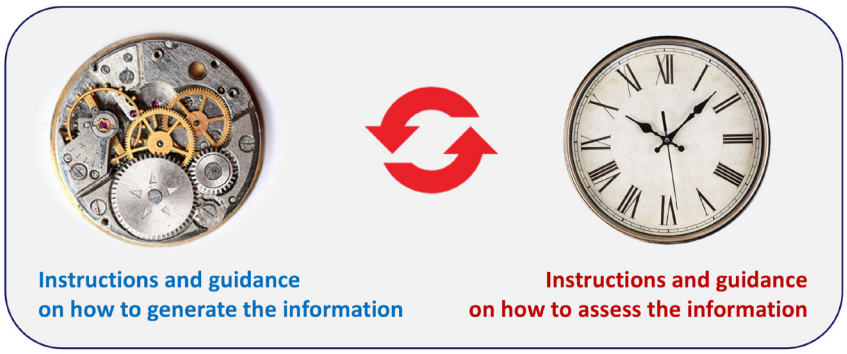

Fig. 4: Exemplification of different perspectives in a single tool

ASPA fulfills various functions such as (i) giving guidance on how to generate NGRA information, (ii) providing a template for a reporting structure, and (iii) giving guidance and transparency to regulators on how to assess the information. This is exemplified by two perspectives of a clock (front and inside).

As mentioned earlier, all major modules that constitute ASPA have previously been defined, for instance within the OECD IATA program (OECD, 2020). A legitimate question is therefore: Does the world need yet another NGRA approach? We will illustrate (i) what is missing in the already available approaches, and (ii) what ASPA provides that makes it different – and potentially more useful – as an NGRA workflow. Prior to the more technical discussion on this matter (below), we provide some background via three parable examples:

Example 1

In 2002, cancer biologist Yuri Lazebnik published the renowned paper "Can a biologist fix a radio" (Lazebnik, 2002). Using an old-fashioned transistor radio as an example (with a limited number of connections and components), he argued that the tools and thinking of biologists differ significantly from those used by engineers. Particularly, the superficial symbolic language of biologists (often drawing boxes with fuzzy definitions and connecting them by lines/arrows without clear connotations) fails to describe a process with sufficient precision to reconstruct it or to exchange a part during a repair process. In contrast, the construction plan of a radio allows any engineer in the world to exchange a part for one of similar function or even to assemble a similarly functioning radio as the original one (though with differing external designs). Biological depictions, however, typically tend to lack quantitative data, detailed connectivity information, exact material specifications, and sometimes even major construction elements. ASPA aims to advance NGRA from the stage of "outlining an assembly of components" towards "a defined and unambiguous workflow that delineates all relevant functional elements as well as their interconnections". Moreover, it provides rules and "gives specifications for the intermediate switches (decision points)" in the workflow.

Example 2

Rockets designed to carry payloads into space exemplify the challenge of constructing a complex system with a robust function. Although all individual parts to build a rocket are known and available, a sizable fraction of rockets still explode after launch. This occurs even though all separate parts (valves, fuel tank, connectors, steering system) were of high quality and have seen extensive validation. A construction plan alone (as in Example 1), created by skilled engineers, does not prevent this from happening. In addition to the plan, an iterative process of learning and optimization is required. The ASPA workflow already now allows iterative processes for a given safety evaluation. Moreover, it is designed to accept future insights from case studies and evaluation runs to further improve and standardize decision points.

Example 3

An object or design may appear different when viewed from a different perspective. Consider a clock (Fig. 4). For some, the clock face, which provides the readout of time, is the key perspective. Others are interested in the back or inside: How is it technically designed? What makes the clock work? What drives its functioning (mechanical or electronic)? Different stakeholders of NGRA have such different perspectives and needs. For regulators, data is a primary objective. This includes their understanding of how to interpret the data. The OECD IATA framework has a strong focus on this. What regulators require is of course also important for data providers, although their focus may be different, and yet other perspectives are relevant for method developers and laboratory scientists. Different stakeholders will have different perspectives on what is of importance or interest. An NGRA workflow like ASPA must cater to all these needs. A related example for different stakeholder perspectives is the Read-Across Assessment Framework (RAAF) from ECHA (Kuseva et al., 2019). It explains how read-across dossiers are to be evaluated, yet it does not explain how read-across is to be carried out, nor what is the best approach to produce a good read-across report. These latter

aspects require additional guidance for the respective stakeholder groups (data providers, submitting companies).

A simplified conclusion, in line with the above examples, is that it is not sufficient to define the individual elements of NGRA (even if this is done very thoroughly). A comprehensive operationalization of NGRA at a high level of granularity has so far remained limited, and progress in this area is essential. There is still an unmet need for a detailed NGRA workflow to practically guide data generation, interpretation, and integration.

4 Overall structure and nomenclature of construction elements of ASPA

Here, a general overview is given, together with a few examples. A full technical review of the ASPA structure is outside the scope of this initial overview; however, the complete current workflow scheme is provided in full detail in Fig. S1[5].

It is helpful for a detailed discussion of some key elements and principles (see below) to introduce some nomenclature. In very simple terms, the flow scheme consists of "boxes" and "arrows". We use the term "building blocks" for all boxes, in analogy to the building blocks of an algorithm, but also following the concept that ASPA is a construct meant to "give NGRA a home". It would not be wrong to view the building blocks as steps in a data documentation process, but we preferred not to use the term "step" for naming, as ASPA has several perspectives, and not all building blocks correspond to steps ahead. The building blocks are also represented in the *ASPA-assist* software platform that guides users through the ASPA workflow. In *ASPA-assist*, as in ASPA, each of the building blocks has a unique identifier and a version tracker in addition to its trivial name.

As with any construction, there can be several types of building blocks (e.g., in a building analogy these would be entrances, bedrooms, bathrooms, corridors, roofing, etc.). ASPA uses six types of building blocks, which are called "basic construction elements" (Fig. 5). They are data generation tasks, operator tasks, decision points, sub-workflows, reporting and problem formulation. In other words: a randomly picked building box from ASPA may be a data generation task or a decision point (or any of the other four).

The basic construction element, "sub-workflow," plays a special role, as it is an assembly of several building blocks. This element was introduced to allow a better overview, as the ASPA v2.1 version has already > 50 building blocks. The next version, currently under development, will have several more. Each basic construction element is defined in the following:

Problem formulation: This element is unique in the sense that there is only one problem formulation in the construction plan. Thus, it is both a construction element and the name of a defined building block. It defines the compound to be evaluated, the regulatory question, the legal framework, the population that is to be protected by the assessment, and (potentially) the use or exposure scenarios of interest. The problem formulation impacts other elements, as it is crucial for parametrizing decision points and determining both the granularity and the level of acceptable uncertainty of information required from data generation tasks and other building blocks.

Data generation tasks: The building blocks representing this basic construction element typically use defined methods to generate new data. The methods used need detailed documentation (e.g., by ToxTemp files (Krebs et al., 2019)), including an assessment of their readiness and performance. The data provided by data generation tasks is directly accessible (e.g., via *ASPA-assist*), and they usually contain links to data repositories. Some of these building blocks make method suggestions or offer direct links to relevant computational methods.

Operator tasks: The building blocks representing this basic construction element require operators to perform an activity. This may lead to knowledge generation by data processing or by the combination of data types, but it usually does not generate data by a defined test method. Examples are the "selection of the most relevant PoD", "performing a biokinetics[8] correction" of nominal concentrations, "defining the set of source compounds for read-across", defining "metabolites that need further investigation" or "defining most-relevant exposure routes". While these tasks are given to human operators and require weight-of-evidence approaches, future ASPA versions are anticipated to automate some of the tasks. For instance, agentic AI approaches may be incorporated into such building blocks (Kleinstreuer and Hartung, 2024).

Decision points (DP) (Fig. 6): The building blocks representing this basic construction element receive input from higher ASPA building blocks and have the single purpose of taking a yes/no decision. Thus, DP are ASPA nodes that lead the evaluation flow towards specific downstream sets of building blocks (dependent on the state of information). DP may require expert judgement (defined by an "E" in the identifier) or they may be automated and data driven ("A" in the identifier). While at present, there are only E-DP, it is likely that ASPA will be increasingly automated and that some A-DP will be operated by AI tools. It is anticipated that reproducibility of ASPA outcomes will be increased if more automated decisions can be incorporated over time. As ASPA is open for iterative processes, DP may steer optimization loops. Therefore, the identifier offers the option of defining them as "O" (one-way) or "L" (loop) DP[9].

As transparency is a foundational principle of ASPA, the rationale and interpretation behind each DP need to be recorded. The rules for decision-making need to be transparent, and the exact way in which these rules have been followed must be documented. Such specifications within DP include: (i) definition of the minimum set of data required for a decision; (ii) definition of criteria to be considered for a decision; this includes guidance on how to weigh them; (iii) guidance on thresholds of effects that are consid-

8 "Biokinetics" is used here to describe experiments (and knowledge therefrom) that investigate the distribution processes of a test compound in a cell culture dish (e.g., intracellular accumulation)

9 Initial ASPA versions used F instead of A, F instead of O, and P instead of L

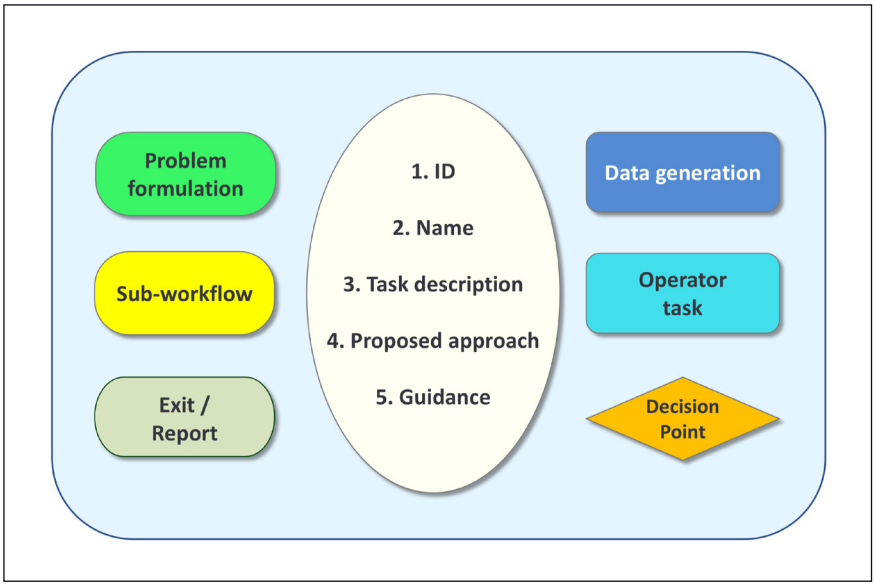

Fig. 5: Overview of basic construction elements of ASPA and *ASPA-assist*
The six types of basic construction elements used in the ASPA construction plan are visualized. Within the *ASPA-assist* software, each of the building blocks built from one of the basic construction elements has five information layers (indicated in the central oval). For an overview of how such building blocks are assembled in ASPA, see Fig. S1[5] (construction of ASPA v2.1).

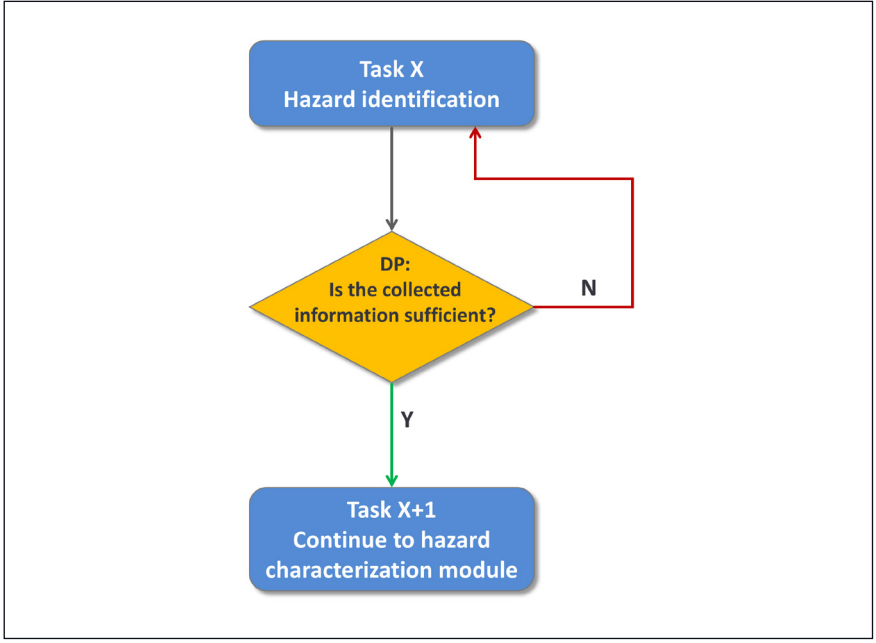

Fig. 6: Exemplification of the function of a decision point working in a loop structure
A typical decision point (DP) between two data generation building blocks is displayed. DP always have two exits. The Y (yes) exit is taken when the answer to the DP question is affirmative. The N (no) exit is taken when the answer is negative (i.e., here, information is not sufficient). DP are switches in the workflow that direct the data collection to particular areas (groups of building blocks) and thus focus the reporting and assessment efforts. Here, the special case of a loop structure is shown, where the DP allows downstream continuation of the flow (Y) or requires increased efforts (and results) in the upstream part (N).

ered relevant; (iv) definition of thresholds of certainty (uncertainty) accepted for each data set/method; (v) rules and formal specifications for the documentation of decisions and decision rationales; (vi) guidance on how to consider historical decisions and how to perform consistency checks.

Sub-workflows: ASPA is organized as a 2D map (Fig. S1[5]), with a limited size and complexity. This allows a good overview but prevents all building blocks being presented in the same way and in plausible relative positions to one another. Instead of creating building block crowding (and potential overlaps) and too many crossing arrows, the overview display uses elements that indicate that a sub-workflow (sub-WF) branches off at a certain point (and would also somehow re-enter the main ASPA plane). The sub-WFs are organized like ASPA itself, using the same workflow building blocks. They include, e.g., the "metabolite investigation WF", the "read-across WF", and the "genotoxicity and topical toxicity WF". This design allows for greater granularity in complex assessment steps without overloading the main framework. In short, a sub-WF is an abbreviation for a conceptually connected set of building blocks. Usually, it contains at least one decision point and one data generation task.

Exit & reporting: There are several ways to conclude and exit ASPA. They include scenarios where no bioactivity is identified or no relevant exposure is expected; or where genotoxicity is identi-

fied and the evaluated compound is thus out of the scope of the initial evaluation goal[10]. Also, some compounds may not be suitable for NGRA as they are outside the chemical and/or biological applicability domain of NAMs (like many volatile, unstable or water-insoluble compounds).

The default exit occurs after the workflow has completed the relevant parts of the main assessment pillars. The reporting and assessment module is still under construction following the evolutionary development principle of ASPA (see below). An ideal outcome of a risk assessment problem could be a human equivalent dose (HED) that marks the threshold to toxicity. This would be complemented by a measure of overall uncertainty. While current ASPA versions rely on expert judgments for uncertainty on various levels, future versions aim to also incorporate semi-quantitative or automated uncertainty characterizations, particularly where validated methods are available. It is envisaged that such information would be converted into a correlate of traditional safety factors. The vision of ASPA is that this information will be used broadly in the future for setting thresholds, such as an ADI (acceptable daily intake), an HBLV (health-based limit value), a DNEL (derived no-effect level) or another benchmark specified in various international regulations concerning safe human exposure. The ASPA structure basically allows for the use of deterministic or probabilistic methods and endpoints, not just for, e.g., exposure assessment or hazard characterization, but also for the overall outcome in risk assessment. The actual application is determined by the problem formulation, the available methodology, and stakeholder requirements (e.g., those of regulators).

5 Major assessment pillars for hazard, ADME, and exposure

For clearer communication, it has proven useful to discuss not only individual building blocks but also larger functional areas of ASPA, which may be referred to as modules. For instance, one major module addresses all aspects of ADME[11] and contains > 10 building blocks. This ADME module may also be called the ADME pillar. The term "pillar" is used as an alternative designation for the three modules responsible for *de novo* data and information generation: ADME, exposure, and hazard (Fig. 2). This terminology is justified because these modules serve as the primary supports of the risk assessment framework within the core of ASPA. Note that ASPA is not limited to *de novo* data generation for risk assessment of compounds. It also allows to choose a read-across approach (in a dedicated module designed according to key publications (Escher et al., 2019; Rovida et al., 2021) where this is suitable (Fig. 4).

If existing data are not sufficient and read-across is not applicable, hazard, ADME, and/or exposure data will be generated within and through interactions among the main pillars.

5.1 Hazard pillar

The assessment of hazard starts with a building block that uses a broad panel of methods to identify bioactivities affected by the test compound. Computational methods (e.g., QSARs, AI-powered data-mining) can play a major role. Some of these methods may trigger alerts for defined traditional toxicological endpoints: genotoxicity, skin sensitization, or acute toxicity (either topical or systemic). ASPA provides for their follow-up in dedicated sub-workflows. Yet, the main focus of the initial ASPA versions is on systemic toxicity after repeated exposure.

Computational methods are complemented by NAMs that provide a broad and rapid overview of potential bioactivities (e.g., reporter assays, cell painting, transcriptomics, pharmacological target interaction panels, cytotoxicity assessment in various cell types). The experimental methods should ensure high sensitivity (but not necessarily high specificity), enabling for instance the provision of a set of PoDs. If no relevant bioactivity is identified and the problem formulation is sufficiently addressed, final conclusions could be drawn and ASPA could be exited at this stage. However, in most cases, compounds will be further examined in a second assessment step. For this purpose, a dedicated building block of the hazard pillar contains tasks to follow up on alerts and to generate plausible links from bioactivity to adverse outcomes, thereby eliminating false positives (increasing specificity) and reducing uncertainties. The integrated information is used to generate a toxicological rationale, i.e., a plausible and relevant chain of events from measured molecular initiating events (MIEs) or key events (KEs; triggered at realistic exposure levels and internally reached concentrations at target sites) relevant for a defined adverse outcome.

It is expected that this procedure will imply the use of increasingly complex (in most cases more resource-demanding) NAMs, e.g., to assess later KEs within a putative AOP. The NAMs applied for this purpose are dependent on (i) the specific problem formulation (including the compound under evaluation) and on (ii) the alerts generated in the previous assessment step. This flexible and tiered approach may also involve iterative loops to generate increasingly plausible (and regulatory-relevant) toxicity information that is eventually robust enough to define a definitive PoD. Using the principle of "*in vitro* kinetic modelling", the nominal concentrations of PoDs can be converted to free concentrations or any other metric to be used for PBK modelling and data integration (Kisitu et al., 2020). Additional experiments may be performed to reduce the likelihood of false-negative predictions.

In all cases, care is taken that the assessed substance is within the applicability domain of the assays (irrespective of whether *in silico* or *in vitro*). Moreover, ASPA stipulates that a relevant PoD can be linked clearly (via a mechanistic rationale such as an AOP) to an adverse effect at the organism level. Under these conditions, PoDs can be used as input for *in-vitro*-to-*in-vivo* extrapolation (in the ADME pillar) to predict a corresponding HED[12] (Chiu, 2017).

[10] Note: Depending on the problem formulation, risk assessors could be interested to learn whether other types of toxicity may occur besides genotoxicity. Then ASPA would be continued.

[11] "ADME" is used here interchangeably with "toxicokinetics".

[12] As defined in (Chiu, 2017).

5.2 ADME pillar

As in the hazard module, data generation in the ADME module proceeds in a tiered manner. In a first tier, an estimate of plasma concentrations of a test compound can be generated from generic, relatively simple PBK models. A default assumption of oral ingestion can be accepted, or alternatives may be chosen, in line with the problem formulation. The conservative assumptions that the compound is 100% bioavailable and that it is only cleared renally (and not by metabolism) will lead to potential overestimates of internal exposure; data on tissue levels will not be available. *In silico* predictions (e.g., on protein binding) are used to parameterize the model with compound-specific input data.

Where required, assessment may progress to higher tiers (implemented in dedicated building blocks) where more refined models can be generated and used. They allow the PBK model uncertainty to be reduced by the generation of experimental data on ADME properties of the test compound (e.g., barrier crossing/transport, metabolism, protein binding, blood-plasma distribution, etc.). Additional data can also be generated on bioavailability and other aspects of ADME. Also, the PBK model structure may be adapted to cover more tissues and life stages (e.g., fetus within mother or young vs old subjects) and to include physiological processes like enterohepatic circulation, renal reabsorption, biliary excretion, and so on. Finally, also the genetic and phenotypic variance in human subpopulations can be considered in the higher-tier building blocks. An important use of PBK models is not only forward modelling but also reverse modelling, i.e., conversion of NAM-derived PoDs from the hazard pillar to HEDs[13].

A special sub-workflow (at the interface of hazard and ADME) deals with metabolite identification and the potential role of metabolites in the overall organismic hazard. Thus, the ASPA design not only accounts for the possibility that some compounds show specific (active) accumulation in some tissues but also considers metabolite-dependent toxicity and ways to identify it (Suess et al., 2025).

Another interface between ADME and hazard provides estimates for top concentrations of testing in the hazard identification building block. For this, ADME models may use compound and case-specific information from the exposure pillar. Alternative inputs are limits defined, e.g., in the Classification and Labelling (CLP) regulation (i.e., 1,000 mg/kg day; EC, 2008). Use of such inputs depends on the problem formulation and the respective regulatory data requirement (e.g., for classification and labelling).

5.3 Exposure pillar

Depending on the stated problem formulation (which specifies the data requirement) and available exposure-related information, an initial estimate of the external exposure is made. The tiered design of this pillar allows stepping from deterministic conservative estimations with low input and high uncertainty towards more refined exposure models such as probabilistic models that account for variations across sub-populations or scenarios. In the initial tier, standardized models are often used, such as ECETOC TRA[14] or ConsExpo[15] for worker and consumer exposure, alongside simple worst-case assumptions (e.g., maximum use frequency, highest concentration). The process also allows for the consideration of already available measured exposure data where possible.

To provide a holistic and aggregate perspective, the exposure assessment is based on real-life exposure scenarios, including all relevant sources and routes. The assessment process considers different environments (occupational, consumer, dietary, environmental), sources (e.g., worker exposure to the same chemical during successive tasks throughout a shift or consumer exposure of the same substance in multiple products), and routes (inhalation, dermal, oral). An important assessment element in the tiered approach is to map the uses of a compound (including (pre-)processing and end-of-life stages) and to screen possible scenarios for human exposure across various settings. The most relevant sources and routes of exposure are then identified and prioritized for further refinement in higher tiers. For example, refinement may involve incorporating more detailed information and applying advanced exposure models such as the ART[16] or PACEM[17]. This allows a focus on the sources and routes that have the highest impact potential on the safety assessment. Thereby, it is ensured that the assessment is complete enough regarding the specifications in the problem formulation, but also that effort and outcome remain balanced.

At the interface of exposure and toxicokinetics modelling, building blocks that investigate barrier metabolism and barrier penetration (e.g., via *in vitro* models or PBK modelling) play important roles.

5.4 Outlook

Examples of higher-tier follow-up assays for hazard, exposure and ADME, and the interconnection of assessment pillars are included in currently running cases studies (e.g., on conazoles, propylparaben, or high-risk chemicals under evaluation by ECHA) (Tab. S1[5]) as well as in previous OECD case studies, e.g., on imidacloprid, valproic acid, and deguelin (Loser et al., 2021; Vrijenhoek et al., 2022; Van der Stel et al., 2021) and in recent publications (e.g., Magel et al., 2024; Meijer et al., 2025).

6 Details in and around the building blocks of ASPA

6.1 Forward- and backward flows

The construction of ASPA uses several basic construction elements[18]. Each building block of ASPA is made up of one of these

[13] Note that various alternative terms for HED are in use in various contexts and regulations. Examples are reference point (RP) used by EFSA, reference dose (RfD), or *in vivo* point-of-departure (PoD), as well as equivalent administered dose (EAD) or administered equivalent dose (AED) used by the US EPA.

[14] https://www.ecetoc.org/tools/tra-main/

[15] https://www.rivm.nl/en/consexpo

[16] Advanced REACH Tool: https://www.advancedreachtool.com/

[17] Probabilistic Aggregate Consumer Exposure Model: https://www.rivm.nl/en/consumer-exposure-to-chemical-substances/exposure-models/pacem

[18] All construction elements are building blocks, but there are several different types of building blocks. In a Lego analogy, every building block is a piece of Lego, but there are different types of pieces (long bricks, short bricks, flat ones, narrow ones, etc.).

basic construction elements. However, ASPA is not just "a pile of building blocks" but rather a well-organized flow-scheme. Therefore, the arrows that connect the building blocks are also key workflow elements. Their interpretation is relatively straightforward at first sight, and much less complex than the key event relationship (KER) of an AOP: They indicate a logical sequence of steps in an algorithmic process of data collection for reporting. Note that this explanation can prevent the following misunderstanding: ASPA is not necessarily the sequence of data being generated. In reality, some of the data generation will often run in parallel, and the real timing of data generation may take weeks to years, while work along the *ASPA-assist* platform for a given case study may take hours to days.

A second and third perspective on the arrows is more complex: (i) Seen from an operator viewpoint, ASPA (in particular, its implementation in *ASPA-assist*), is a traceable and transparent pathway of recording data and of explaining decisions taken during the data gathering, interpretation, and integration process. This is done with a particular focus on providing transparency and traceability to an assessor. For instance, during compound evaluation, many iterative experimental steps may be included. This means that experiments may be re-run within a building block (e.g., for hazard identification) to obtain more accurate, reliable and robust information. It is also likely that there will be many experiments designed as plausibility, quality and completeness checks. Some experiments may have to be redone, with changed setups, different replicate numbers or other variations to come to final robust conclusions, as one learns more about the test compound and its toxicological behavior. (ii) Seen from an assessor/regulator viewpoint, arrows will often have to be followed in the reverse direction. The starting point is the final outcome (e.g., a suggestion for an HED and a quantification of uncertainties), and questions will arise as to what the underlying rationale is and what the final data are. ASPA can be used for backward tracing towards the origin of data or zooming in to understand the rationale for why certain issues were considered, or neglected, and how gaps can be explained or justified, and how alerts were followed up. ASPA helps to move backwards to see whether and how overall conclusions are justified and supported by the outcomes of major modules and whether these are supported by outcomes from individual building blocks. Only on a third level may all the highly technical, often tiered, and sometimes iterative steps within all building blocks be of interest. For this purpose, ASPA ensures that all critical aspects of risk assessment are addressed in a scientifically coherent manner and can be easily evaluated.

The ASPA workflow allows new information to be integrated once it becomes available or if certain assumptions must be revisited: the workflow allows adaptations and also tracing backwards (opposite direction of the arrows), refining/updating the output from a particular building block and re-running the downstream parts towards risk assessment.

6.2 Guidance for users and operators

In addition to considering the 2D structural representation of ASPA, one can envision a third dimension for each building block. Besides name and unique identifier in 2D, several layers of information and guidance are available, including:

Task description: Gives a focused, high-level description in no more than three sentences.

Proposed approach for completing the task: Some recommendations are given. They may address the level of detail and quality of data, and may suggest approaches and methods. These are not prescriptive, but exemplary. Overload is avoided but examples help with understanding the scope of a building block. There may also be indications of which sub-tasks are meant to be included within a task and what is expected to define the building block as accomplished.

Guidance: Here, a more detailed description of what is expected as outcome is given. The overall task/module is broken down into defined sub-questions that comprise all aspects of what could be considered and provided. The type and level of outcome is defined in detail. This comprises the scope of testing, a definition of quality expectations, a metric for uncertainty of results, a documentation of statistics and confirmatory assays, etc. Where sub-questions alone are insufficient, explicit examples of what is meant/required are given. This may also include statements on what is not meant/not required. In some cases, alternative options that would be possible or acceptable are indicated. Examples will initially be biased towards methods used in the ASPIS cluster projects, but alternative approaches that yield similar information are explicitly encouraged. An important aspect of the guidance is the cross-linking to regulatory guidance already available and relevant for respective NAMs or building blocks. Examples are the reporting frameworks for PBK models, for omics data, and the OECD guidance documents on QSAR assessment (OECD, 2023a,b), as well as a number of guidance documents or guidelines from EFSA or ECHA. Any additional material assembled by national and international bodies and expert groups may be considered (Pamies et al., 2022; Keßel et al., 2023; Hartung et al., 2024).

It is planned to document this third dimension within each building block in a webpage format, where it can be easily updated and where links may be provided to relevant (and sometimes extensive) guidance documents without overloading the ASPA workflow itself.

7 ASPA development – an evolutionary process[19]

The development of ASPA was initiated within the ASPIS research cluster and was, from its conception, designed to become a community project involving all relevant stakeholders. Initially, a concise toxicity domain was covered from which the workflow could be further expanded in a modular way. Chronic systemic toxicity – aligned with the objectives of the ASPIS projects – was

[19] The term "evolutionary" is not meant in the sense used in classical biology (heritable change of a species by random mutation and natural selection). It is used here in the everyday language (colloquial) meaning of "gradual development and improvement over time", in line with the ever more complex and detailed new versions.

the initial target, but the framework is readily adaptable to other endpoints and exposure conditions. This versatility supports greater harmonization across jurisdictions and use cases. At present, the information requirements for some toxicity areas (relevant to systemic toxicity) have not been implemented in ASPA. This applies to, for instance, bone marrow toxicity, hematotoxicity, and immunotoxicity, and to some organs (e.g., reproductive system or heart). Inclusion of such areas into the NGRA workflow is not a fundamental technical limitation but merely reflects the deployment of available resources at the early implementation stage of ASPA to achieve proof-of-concept.

The evolutionary development approach is reflected in the versioning of ASPA. The initial concept (v1.0) has been discussed and refined at several workshops and ASPIS meetings since 2023. An intermediate stage was reached with v1.9, which was implemented in a software tool (*ASPA-assist* 1.9), which formed the basis of several case studies, and which was discussed in detail with regulatory and industry stakeholders in May 2025 (ASPA-NGRA Workshop, BfR, Berlin). Further developments led to v2.1 (Fig. S1[5]) as the next consolidated stage (used for OECD case study submissions in 2025). Currently, work is focusing on v3.0 (initial outline to be presented at the EUROTOX 2025 meeting in Athens), which is the first version intended to go fully public.

Both the regulatory applicability of ASPA and its actual use in a regulatory context are likely to evolve over time. Initially, ASPA may be tailored according to the needs of different regulations and used in weight-of-evidence approaches to complement or even substitute information requirements that are currently primarily met by guideline animal studies. Possibly, there will also be a learning and confidence building phase of parallel usage of traditional studies and ASPA. Part of the evolutionary process will be the use of ASPA by various stakeholders in case studies across different sectors and regulatory jurisdictions to provide a basis for further optimization. Subsequent steps include more formal validations of certain building blocks and modules in terms of relevance and robustness. For instance, a key question to inspire confidence into ASPA-based safety evaluations will be the experience with the metabolite sub-workflow. The ambition for ASPA in its final form is to provide a NAM-based information equivalent to, e.g., sub-chronic repeated dose 28- and 90-day toxicity studies under OECD TG 407 and TG 408 (OECD, 2025a,b), or developmental neurotoxicity (DNT) studies (TG 426) (OECD, 2007), or specific neurotoxicity (TG 424) (OECD, 1997) or carcinogenicity studies (TG 451; TG 453) (OECD, 2018a,b). A long-term vision is to use ASPA as a building block for an overall novel NGRA strategy that is protective for the human population but does not necessarily substitute the current animal-based system on a 1:1 basis.

Last, but not least, an aspect of continuous ASPA evolution is its sustainability after the end of the ASPIS project cluster. Several mutually non-exclusive options are being pursued: (i) further development within other large public projects such as PARC (De Castelbajac et al., 2023; Marx-Stoelting et al., 2023; Herzler et al., 2025b); (ii) implementation at a sustainable risk assessment institution (e.g., EFSA); (iii) transfer to a commercial platform or to several contract research organizations; (iv) creation of a governance body responsible for further development and auditing of official versions, possibly in the style of the EBTC collaboration[20], the MPS Society steering board[21] or the Alternatives Congress Trust (ACT)[22].

8 What ASPA is and is not

ASPA is a workflow designed for safety scientists to document their methods, input results, interpret findings, and justify decisions; it is also an assessment tool for assessors and regulators, to help them in the data evaluation process that leads to risk assessment. ASPA ensures that all relevant building blocks are considered and provided with information (i.e., all respective "boxes" to be filled in *ASPA-assist*). This way, it encourages a disciplined and methodical process and provides traceability of the flow of information. Decision points with binary options (e.g., Is the PBK model prediction acceptable?) direct the user to the next appropriate assessment step based on the chosen response.

Prior to compilation of information into this review, several communication channels have been used to describe ASPA, including:

(i) a series of stakeholder workshops/conference sessions (four in 2025, more to follow in 2026);
(ii) newsletters and video demonstrations that can be found on the RISK-HUNT*3R* project website[23];
(iii) access of workshop participants and other interested stakeholders to the user interface that allows the entering of own case studies in ASPA format (*ASPA-assist*). This enables a practical and intensive first-hand contact and exploration (contact details on RISK-HUNT*3R* project website[23])
(iv) exemplification of ASPA by ASPIS case studies in the context of the OECD IATA case study program (3 submissions in 2025, more to follow in 2026).

8.1 To avoid misunderstandings of the above, here is what ASPA is not:

ASPA is NOT a one-click risk assessment[24]: It is not meant to be an automated process and will typically require expert judgement for decision-making in many cases. Moreover, in most cases, generation of experimental data is likely to be required.

ASPA is NOT a super-QSAR. Instead, it guides and documents the process of doing NGRA with data from multiple sets of experimental NAMs and computational models. It requires experimental

20 https://www.ebtox.org/

21 https://impss.org/about-us/

22 The ACT organizes the world congresses on alternative methods. See here for reference: https://www.wc13rio.org/about/

23 https://www.risk-hunt3r.eu/

24 This means that ASPA does NOT "take decisions"

(and *in silico*) data generation AND specific steps for data integration AND multiple decisions by sufficiently knowledgeable operators.

ASPA does NOT circumvent the main issues of NGRA as such; it "only" aims to make it more transparent and reproducible. It likely requires extrapolation from MIE/KE to AO. It may require toxicokinetic modelling and prediction. It may require selection and integration of high-dimensional datasets. It works better with "protection objectives" than for producing "defined predictions of specific adversities".

ASPA is NOT a defined toolset. Instead, it defines the type of information required (it is technology-agnostic; i.e., it specifies information requirements while leaving the decisions on how to obtain the required data sets to the operator). It makes recommendations/gives examples for suitable technology choices and specifies a frame of validity and uncertainty required.

ASPA is NOT a tool to assess the reliability and relevance of NAM methods for a certain endpoint/problem formulation. ASPA does stipulate that such information be provided together with the data in the form of a method documentation that includes data on its readiness state, the method performance, and an overview of, e.g., relevant controls for the method endpoints (Krebs et al., 2019). The choice of the NAMs, including their applicability domain and their suitability to provide the required information, will be assessed in ASPA within the weight of evidence and uncertainty building blocks in the risk assessment module.

Last, but not least, ASPA is NOT static (see Section 7). By its modular design, ASPA can be updated to integrate, e.g., new regulatory data requirements, new validated NAMs, new test guidelines, new interpretation procedures, or new modelling approaches. Consensus meetings, exemplified here for DNT, may form a basis for this (Celardo et al., 2025; Cöllen et al., 2025).

9 *ASPA-assist* on the NAMASTOX platform

While ASPA as a schematic workflow may be a useful theoretical concept, its practical application can be demanding for the toxicologist, requiring a deep understanding of the process and a high degree of discipline to document all the workflow steps. To address this issue, a software tool has been developed that guides users stepwise through the entire workflow. It supports the collection of information, by specifying, at each step (for each building block), which type and extent of information should be entered, or how decisions should be explained and justified. Guidance on how to proceed is given in five information layers within each building block (Fig. 5). It also directs the user towards the next relevant building block, depending on the information entered in previous building blocks. Thus, users do not need to know or memorize the exact construction plan of ASPA but simply provide (or control) the data, following the flow given by the software. Total flexibility is given by an ASPA overview map and the possibility to jump to any point in ASPA.

This *ASPA-assist* tool, mirroring the ASPA architecture, brings ASPA to life through an intuitive graphical user interface, accessible online or offline through a web interface. *ASPA-assist* was implemented based on the generic workflow tool NAMASTOX (Pastor et al., 2024), and different versions were developed to operationalize specific ASPA versions (e.g., *ASPA-assist* 2.1 implements ASPA workflow v2.1). The latter version can already be accessed by a select group of stakeholders, and this may be expanded upon request. *ASPA-assist* 3.0 should be the first fully open-access online version. In parallel, the same tool is planned to be made available to companies, which can install and run it locally (behind their firewalls or even on isolated desktop computers).

A detailed description of *ASPA-assist* will be provided elsewhere. For a general impression, a video illustrating its use is available[25]. An interesting feature of *ASPA-assist* is its ability to automatically generate reports. They compile all NAM data, as well as the justifications for all intermediate workflow decisions, in structured documents suitable for various regulatory audiences. In the future, users will be able to select reporting templates aligned with requirements by different authorities, such as EFSA, ECHA, EMA or OECD. Integration with platforms such as IUCLID is under discussion. These outputs help ensure traceability and regulatory acceptance. Additionally, each building block can be re-visited to consult the original data (with links to raw and metadata) and to obtain information on methods used (e.g., from ToxTemp files (Krebs et al., 2019), on method readiness, and on uncertainty documentation.

Data automation is an area of ongoing development. For certain task boxes, especially those involving *in silico* predictions, *ASPA-assist* can submit the substances to predictive tools (e.g., QSAR tools) and automatically collect the results and model documentation. The goal is to expand automation further, particularly for data retrieval and structured interpretation.

10 Exemplary case studies

As for most complex tools, hands-on experience and test runs are the best way to understand ASPA's function and applicability as well as to identify shortcomings and gaps of initial versions.

For this reason, several case studies were performed, following the ASPA format (Tab. S1[5]). Three of them have been submitted to the OECD IATA case study program (currently under review), and several more are in preparation for submission in 2026.

Details on the picoxystrobin evaluation are already publicly accessible (Magel et al., 2024), and we therefore use this case study to briefly exemplify the ASPA workflow for a risk assessment problem formulation. The test compound is a fungicide belonging to the strobilurin class. The problem formulation defines the scope: Evaluation of the potential hazard of picoxystrobin for DNT when it is present as a contaminant on imported fruits (Note that this is not the full scope of a new pesticide registration in the EU as it would be evaluated by EFSA). For purposes of the case

[25] https://youtu.be/rSVbWoSQssc

study, it was pretended that the available hazard data were insufficient (unknown), and that neither TTC (threshold of toxicological concern) nor read-across were applicable.

The ASPA workflow was used to address the question: Does fetal exposure fall within a range that could cause adverse effects in humans? For exposure estimation, oral intake was considered as the primary exposure route. Using available dietary exposure data, a maximum external exposure of 0.09 mg/kg/day was calculated. Since detailed ADME data were lacking, *in silico* and *in vitro* tools were used to parametrize the PBK model. These included barrier transition models and clearance assessments. PBK modelling then provided internal exposure estimates for both maternal and fetal tissues, including the brain.

Next, the hazard potential was evaluated. *In silico* screening flagged picoxystrobin as a potential mitochondrial toxicant. This alert was followed by a tiered *in vitro* hazard evaluation, including general screening and dedicated assays for DNT. One assay, focused on neural crest cell migration, showed a significant effect independent of cytotoxicity, warranting further investigation.

Subsequent steps involved confirming the hit, using different methodologies, and refining the NAM-based PoD for relevance to long-term/repeated exposure. Mechanistic studies validated mitochondrial inhibition by picoxystrobin. Biokinetic modelling predicted the compound's accumulation within cells by a factor of up to 100-fold. This allowed for correction of PoDs and ensured relevance to real-world exposures.

Finally, internal exposure levels were derived from PBK modelling, and they were compared to PoDs obtained from the *in vitro* studies. This comparison suggested an exposure-hazard ratio of ≥ 80 as an input for risk assessment. This means that the HEDs were 7.2 mg/kg/day or higher. *ASPA-assist* documented each decision point, assumption, and uncertainty along the way.

Case studies like these show the promising potential for the use of the ASPA workflow and *ASPA-assist* tool in supporting complex systemic toxicity evaluations for target organ toxicity, DNT or non-genotoxic carcinogens.

11 Conclusions and outlook

The fundamental information modules for NGRA have been clear for over 15 years, supported by various case studies demonstrating their application in risk assessment (Tab. 1). Documentation and reporting requirements have become increasingly detailed, for example, in the OECD IATA program. ASPA is fully aligned with this existing knowledge and logic. Compared to some other approaches, it may place greater emphasis on external exposure data and the relationship between internal and external exposure. However, the truly novel element is not in the data itself but in (i) the reproducible and traceable process of data integration, (ii) the structured method of obtaining and documenting information, and (iii) the straightforward, highly defined backward traceability from a final data set used for risk assessment to its sources and the methods applied to generate it. This is expected to render data reporting and risk assessment more structured, thereby enhancing communication and understanding between data providers and assessors. It is anticipated that ASPA will serve as a tool to bolster confidence in NGRA and facilitate its broader adoption while still allowing users the flexibility to rely on their preferred methods and established practices for generating the input required by the ASPA modules.

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Conflict of interest

Several of the authors are employees of companies or organizations that deal with chemical risk assessment. Some are directly or indirectly rewarded for work on improved risk assessment strategies. As such, this may be a perceived or real financial interest in the outcomes of the research and the development of ASPA. The authors affirm that their contributions to the research and the manuscript were conducted with scientific integrity and without bias influenced by their association with their organizations.

Data availability

All data are being made freely available upon request.

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