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Points of Reference

Special Series

Animal-free Safety Assessment of Chemicals: Project Cluster for Implementation of Novel Strategies (ASPIS) definition of new approach methodologies

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Points of Reference are part of a regular series intended to address an emerging or controversial topic of interest to the scientific community.

Since the release of the U.S. National Academy's report calling for toxicology to evolve from an observation-based to a mechanism-based science (National Research Council, 2007), scientific advances have shown that mechanistic approaches provide a deeper understanding of hazards associated with chemical exposures. New approach methodologies (NAMs) have emerged to assess the hazards and risks associated with exposure to anthropogenic and/or nonanthropogenic stressors within the context of reduce, refine, and replace (the 3Rs). Replacement refers to achieving a research goal without using animals. Reduction means applying methods that allow an investigator to obtain comparable information and precision using fewer animals. Refinement refers to changes in procedures that decrease or eliminate the animals' pain, stress, and discomfort both during experimental procedures and in their daily social and physical environments (Russell & Burch, 1959). The development, acceptance and implementation of NAMs has become an international priority for human health and that of wildlife and ecosystems. The global commitment to nonanimal research is driven by societal values on animal welfare and the uncertainty of mammalian model species as reliable human surrogates. In addition, NAM-based information can potentially unite the different branches of toxicology by its relevance in protecting human health, wildlife, and ecosystems, thereby contributing to public safety, ecological resilience, and sustainability.

Several international agencies concur that NAMs comprise any technology, methodology, approach, singly or combination, that provides information on chemical hazard and risk assessment allowing for a reduction in traditional animal testing. The NAMs acronym however, has been interchangeably used to indicate "new approach methodologies" and "nonanimal methods." This dual meaning has created discord among groups that ultimately strive to safeguard against exposure to harmful substances without the use of animals. Because an official definition of a NAM has not been established, debates continue on what is acceptable as a NAM, especially for those groups that prioritize reduction over replacement. Furthermore, the idea that a NAM should contribute to the eventual elimination of animal testing is not universally accepted.

A NAM can be defined by the technologies and cognate data that offer information about chemical modes of action or exposure thresholds expected to produce adverse health effects. Thus, any technology that can contribute mechanistic knowledge in determining protection levels for human and environmental health is defined as a NAM, even if the technology uses animal models (i.e., reduction). In contrast, nonanimal methods are primarily defined by testing platforms that avoid the use of animals. In this case, NAMs are test systems; including biochemical and in vitro assays, as well as those using nonanimal species, as defined in Directive 2010/63/EU (i.e., replacement). Animals are defined in the consolidated text of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes EU Directive (2010). They are all nonhuman vertebrates, which includes *Xenopus* and *Danio rerio*, cyclostomes, cephalopods, independently feeding larval forms, and fetal forms of mammals from the last third of their normal development. In relation to this final point, zebrafish embryos do not to fall within the protected life stage for animals until Day 5 postfertilization, so before Day 5, they form an alternative to animal toxicity testing (EU Directive, 2010).

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To address this discord, the Animal-free Safety Assessment of Chemicals: Project Cluster for Implementation of Novel Strategies (ASPIS) provides a unique opportunity to provide an inclusive NAMs definition to help guide this discussion. The ASPIS is a collaboration for research and innovation among three consortia with distinct foci: ONTOX (Vinken et al., 2021), PrecisionTox (PrecisionTox Consortium, 2023) and RISK-HUNT3R (Pallocca et al., 2022). It is part of the European investment in developing nonanimal solutions for chemical safety assessment. Through its complementary assortment of mechanistic data obtained using 3Rs-compliant platforms, including in vitro, in silico, and nonanimal models, it proposes to classify NAMs for regulatory purposes. Additionally, NAMs should contribute to eliminating animal testing by exploring a battery of alternative models to provide toxicological information than the current apical endpoints.

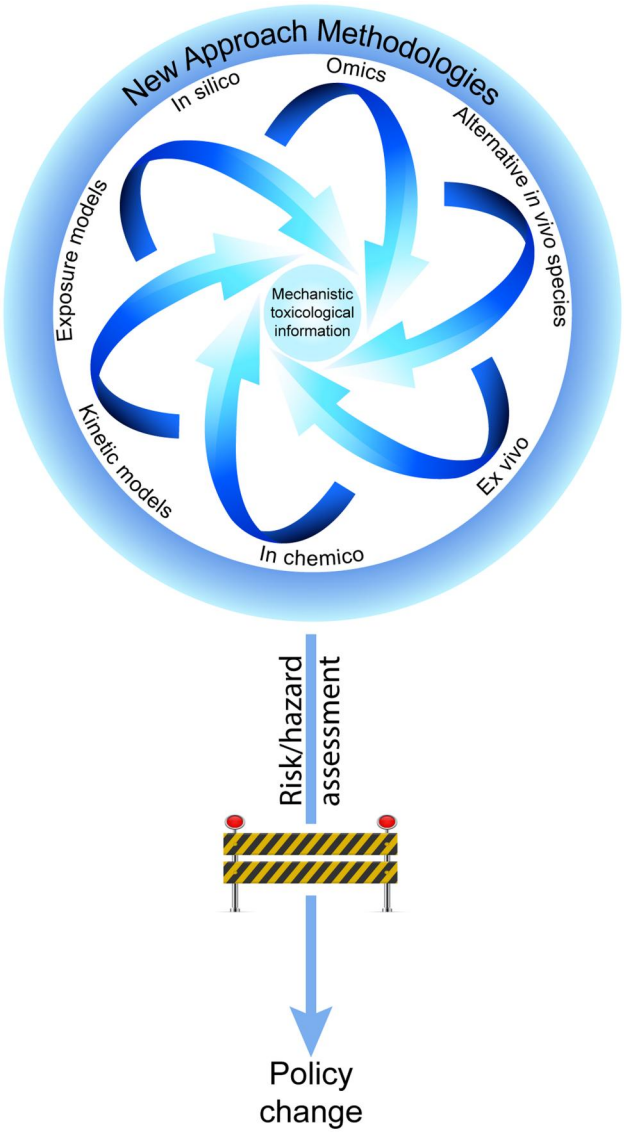

Figure 1. Animal-free Safety Assessment of Chemicals: Project Cluster for Implementation of Novel Strategies (ASPIS) definition of new approach methodologies (NAMs) leading to policy change. New approach methodologies are technology-agnostic approaches that provide mechanistic toxicological information. This information is used for hazard and risk assessment. As barriers inhibiting the application of these methods are removed, NAMs can be utilized in chemical safety decisions.

Any NAMs definition needs to take into account the regulatory regimes within which alternative approaches may develop. Depending on the toxicological endpoints, NAMs have to provide two types of information: the threshold below which an exposure is considered safe and the mechanisms of toxicity/modes of action. These can ultimately be used to understand how exposures lead to adverse responses and pathologies. Although considerable barriers exist that inhibit the application of NAMs in regulatory toxicology (Cavoski et al., 2024), their fundamental purpose is to inform regulatory decision-making while reducing reliance on conventional testing requirements (Figure 1).

The ASPIS NAMs definition was produced through discussions with its membership, Scientific Advisory Group, and from presentations for public comment. Based on this input, ASPIS currently defines NAMs as approaches that provide mechanistic toxicological information; based on exposure, toxicokinetic, toxicodynamic, and molecular toxicology; for human and environmental hazard identification and characterization that contribute to animal-free next generation risk assessment. This definition will likely evolve by the conclusion of the three projects.

Two complementary approaches are also under development as part of the application of NAMs in regulatory hazard assessment: predictive and protective. Predictive NAMs data can enhance the understanding of toxicological mechanisms and are used in the context of adverse outcome pathways to infer the negative consequences that can be expected in an organism. The types of adverse responses and dose at which this effect occurs, based on in vitro and in vivo extrapolation. Protective approaches utilize a variety of NAM models to characterize the toxicological potency of a compound. In vitro and in vivo extrapolations are then applied to derive a threshold below which the chemical of interest does not alert for a critical interference with biological processes. Within the scope of NAM testing, the definition of critical interference needs to be further developed.

In conclusion, the evolution of regulatory toxicology from observation- to mechanism-based science has led to the development of NAMs. New approach methodologies aim to assess chemical hazards and risks without relying on animal testing. The ASPIS is developing essential methodologies for replacing animal testing while maintaining risk assessment accuracy by providing a working definition that blends mechanistic data with appropriate test systems.

Author contributions

John Colbourne (Conceptualization, Funding acquisition, Writing—original draft), Sylvia Escher (Conceptualization, Writing—original draft), Robert Lee (Conceptualization, Writing—original draft), Mathieu Vinken (Conceptualization, Funding acquisition, Writing—review & editing), Bob van de Water (Conceptualization, Funding acquisition, Writing—review & editing), Jonathan Freedman (Conceptualization, Writing—original draft, Visualization)

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Conflicts of interest

The authors declare no conflicts of interest related to affiliations, relationships with funding sources and any other financial or management relationships.

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