

Risk assessment in the 21st century: where are we heading?

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Reliance on animal tests for chemical safety assessment is increasingly being challenged, not only because of ethical reasons, but also because they procrastinate regulatory decisions and because of concerns over the transferability of results to humans. New approach methodologies (NAMs) need to be fit for purpose and new thinking is required to reconsider chemical legislation, validation of NAMs and opportunities to move away from animal tests. This article summarizes the presentations from a symposium at the 2022 Annual Congress of the British Toxicology Society on the topic of the future of chemical risk assessment in the 21st century. The symposium included three case studies where NAMs have been used in safety assessments. The first case illustrated how read-across augmented with some *in vitro* tests could be used reliably to perform the risk assessment of analogues lacking data. The second case showed how specific bioactivity assays could identify a NAM point of departure (PoD) and how this could be translated through physiologically based kinetic modelling in an *in vivo* PoD for the risk assessment. The third case showed how adverse-outcome pathway (AOP) information, including molecular-initiating event and key events with their underlying data, established for certain chemicals could be used to produce an *in silico* model that is able to associate chemical features of an unstudied substance with specific AOPs or AOP networks. The manuscript presents the discussions that took place regarding the limitations and benefits of these new approaches, and what are the barriers and the opportunities for their increased use in regulatory decision making.

Key words: NAMs; NGRA; safety assessment; AOPs; computational toxicology.

Introduction

The last two decades have witnessed a biotechnology revolution, thanks to marked developments in computing and molecular biology, and the explosion of tools for assessing perturbations of test systems by chemicals and other stressors. This has led to rapid advancements in *in silico*, *in chemico* and *in vitro* methods for the assessment of the hazards and risks of chemicals, which fall under the term of new approach methodologies (NAMs).

NAMs are defined as any technology, methodology, approach or combination that can provide information on chemical hazard and risk assessment, and avoid the use of animals.^{1–3} NAMs include *in silico* and modelling tools, artificial intelligence and machine learning systems, high-throughput data, bioactivity *in vitro* assays, toxicogenomics-based tests, *ex vivo* systems, read-across and grouping approaches, microphysiological systems (e.g. organs-on-the-chip) and *in vitro* to *in vivo* extrapolation (IVIVE).⁴

Reliance on animal tests for chemical safety assessment is increasingly being challenged, not only because of ethical reasons that make these approaches unacceptable to large parts of society, but also because they procrastinate regulatory decisions and because of concerns over the transferability of results to humans.^{5–7}

Therefore, NAMs have the potential to improve safety assessments by using more human-relevant tools through coverage of

key biological pathways or targets. Next-generation risk assessment (NGRA) provides a way to integrate NAM data from various sources into the decision-making process.

NAMs can mimic human biology and provide mechanistic information about how a chemical may cause toxicity in humans. The mechanistic information can be organized into adverse-outcome pathways (AOPs) and AOP networks.^{8–12} The AOP framework connects through causal relationships an initial exposure event to a series of mechanistic and testable key events (KEs) and ultimately to a clinically relevant disease outcome. When AOPs are augmented with concentration- or dose-response data for the different KEs in the pathway, they can be converted into fully quantitative models of toxicity.¹³ AOPs for numerous toxicity effects have already been developed.¹⁴ However, as more and more AOPs and quantitative AOPs (qAOPs) for many more apical endpoints are established, it may be possible to predict adverse outcomes (AOs) and their safe doses in an increasingly expanded toxicological space, by measuring molecular or cellular events *in vitro* at lower levels of biological organization without whole animal testing.

Although *in vitro* testing within qualitative AOPs will permit prediction of complex hazard endpoints (hazard identification), qAOPs will additionally predict the associated safe doses for risk assessment. A powerful tool for converting a qualitative AOP in a

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